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REGENERATION[1]

INTRODUCTION

J. WILLIAM BUCHANAN
NORTHWESTERN UNIVERSITY

SELF-REPAIR characterizes all protoplasm. It is a fundamental perquisite in adjustment to the environment provided by a violent world. Self-repair may be occult, involving de-toxification and restoration of intra-cellular components, or patent, involving reconstruction of lost structure. In the latter we may distinguish rather sharply between structural stoppage of the wounded region which preserves more or less vicariously the wholeness of the organism, and the restitution of the lost structure in a form both structurally and functionally complete. The latter, regeneration in its strict sense, is presented for consideration here. Except to the student of the phenomenon, the dramatic character of regeneration is often overlooked. For not only is the process *dramatic* in many cases, but so obvious in its outward manifestations and so secretive in its fundamental processes as to excite the imagination and to defy analysis. For example, the forelimb of a salamander is cut off. Immediately and before the unaided eye the wounded animal proceeds to demonstrate with startling boldness many of the major problems which preoccupy the student of developmental physiology. Cells migrate and are mobilized at the wound area,

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ingesting moribund and destroyed tissue. Under some as yet unknown influence, cell divisions begin and a white blastema quite readily visible to the unaided eye very soon appears. Subsequently, this blastema elongates, and presently the several tissues of the normal limb become differentiated. With unvarying accuracy the elongated structure assumes the characteristics of the member removed. The phenomenon of polarization becomes evident; symmetry appears, and growth continues until within several months the newly formed limb attains the functional and structural equivalence of the former limb. Few materials in all biology demonstrate with such completeness and on such a large scale cell division, growth, differentiation, symmetry, polarization and the establishment of function. The observer is challenged by this performance, yet the controls operating in the large-scale events taking place under his most careful scrutiny are so elusive that thus far they have not been stated with convincing clarity. True, it is possible to perform certain measurements and to describe the process in terms of change of size and change of condition with time. It is also possible to describe with considerable accuracy the order in which the several tissues and parts of the new limb appear and to state the obvious fact that these structures have the high respiratory rate and high susceptibility of young tissue. But as yet there has been laid before us too little that convincingly demonstrates the physiological controls upon which development depends.

What is true of our knowledge of the fundamentals in the regeneration of the limb of the salamander is also too largely true of our knowledge of regeneration in other forms of animal and plant life. But effort that has been perennial since the time of Spallanzani has produced an enormous literature from which may be culled much that is helpful.

Regeneration may be considered both as a problem in itself and as a technique or approach to the study of more general problems. Valuable indeed is the study of the

potentials and course of regeneration in the various levels of animal complexity, but we are not greatly aided in the solution of its fundamental processes by such data. For it is often found that the capacity for regeneration varies very widely between rather closely related groups. For example, the capacity for regeneration in the polypharyngeal flatworm, *Phagocata gracilis,* may be described as high, fragments as small as one-sixteenth ofttimes regenerating the whole. But in *Procotyla fluviatilis,* a member of a genus so closely related that the exact position is still a matter of dispute, regeneration potential is confined to the anterior third of the animal; section in more posterior regions results in a simple form of wound healing.

Similarly important are the changes in regenerative capacity with changes in age of the animal. Although the generalization may be made that the capacity for regeneration decreases with age, there are certain confusing exceptions. For example, in certain ascidians cleavage is highly determinate, isolated blastomeres being incapable of reconstituting the whole, yet the adult is capable of extensive regeneration.

Most attractive and suggestive are the evidences and data on fundamental biological problems obtained in studies on regenerative phenomena, for in every case of regeneration the investigator is faced by an amazing variety of problems. What consequences of injury or of isolation are responsible for the mobilization of the repair mechanism? What induces the accompanying cell divisions? What cells are involved—specific regenerative cells or tissue cells that in the uninjured normal animal perform quite other functions? Is differentiation thus a reversible process and do the cells involved in formation of the regenerate de-differentiate prior to reorganization of the lost part, or are residual undifferentiated or plastic cells solely responsible for repair? What are the influences that induce differentiation of the several new tissues in the regenerate? What controls the spatial arrange-

ment of regenerating parts? By what means is the new or restored part limited in growth and organization to replacement of the lost part? For only occasionally does hyper-regeneration occur. And towering above all these and other problems of detail is the immensely greater problem of integration of the individual.

Since the time of Plato much has been said concerning the integrating mechanisms that convert an aggregate of individuals into an organism of higher order with properties and potentialities not predictable in the qualities of its members. The Gestalt theory is one modern form of the concept. Too often metaphysical influences have been prematurely called into being to explain individuation and too often these are flavored with vitalism. The integrations of animal societies have recently been brilliantly reviewed by Emerson. It is, of course, unthinkable that the mechanisms of integration of the cells that compose the biological individual are identical with those operating in a group of individuals. Nor is there any logical necessity for assuming that integrating influences which confer wholeness are identical in different types of animals.

In form development Wilhelm Roux drew the exact geometrical centre of the problem of integration in his generalization that the developmental fate of a cell is influenced by its position with reference to other cells. Whitman likewise in his remarks on the inadequacy of the cell theory calls attention to the importance of the spatial relations of cells. Tremendously valuable contributions illuminating the mechanism of organismic integration have been made in recent years by the experimental embryologists.

But nowhere else is the principle of individuation more strikingly expressed than in regeneration. In miniature, reconstitution in Protozoa expresses the organization factors. In the re-association of cells of the lower invertebrates integration and rise of the new individual are necessary complements. The organization of a new and complete animal from a fragment of a flatworm or an annelid implies again the principle of individuation.

At this point regeneration blends with the phenomenon of agamic reproduction. Briefly stated, agamic reproduction involves the property of reconstituting a complete individual from a single cell or group of cells and implies the property of developing the leading, dominant, or organizing element which assumes control both morphologically and physiologically, in the integration of the regenerate. Thus the study of regeneration ofttimes becomes an important approach to the study of the physiological states responsible for agamic reproduction, so common in plants and widely employed among animals. Here again the nature of the organizing and maintaining influences in individuation becomes the goal.

A mechanistic interpretation of organizing action requires that the implements of individuation be physiological in character. Although we have recently been discouraged by R. S. Lillie's scholarly analysis arriving at the conclusion that we can not hope to know the nature of the organizing principle, we have been encouraged by the adduction of certain evidences of knowable controlling factors. One, and perhaps the more widely held view, is that organismic control is established and maintained by reason of the diffusion of specific organizing substances arising as the result of specific metabolisms of organizing or inducing centers. Much confirmatory evidence has been obtained by experimental embryologists, yet there is no general agreement as to the fundamental nature of such substances nor as to their action on cells and tissues brought under control. What is still more important and fundamental in such a concept of the nature of induction, namely, the accounting for the apparent fact that organizers attain a specifically qualitatively different metabolism from that of subordinate regions, is, in general, omitted from the theory. As yet, regeneration has afforded no direct evidence of the existence of organizing substances. If they are present in an isolated fragment of a planarian, we must account for their origin at one particular region of the piece, namely,

that portion which later regenerates or reconstitutes the head. Since all regions of such a piece are capable of producing a head and all are subjected to injury and isolation, but only the anterior end reproduces the head, it becomes exceedingly difficult to apply the organizer concept implemented by organizing substances to head regeneration in planarians.

A second interpretation of the nature of the organizing action relates it to primary quantitative differences in protoplasmic activity between dominant and subordinate regions. Considerable evidence in support of this interpretation has accumulated from studies on regeneration, particularly in Coelenterates, Planaria and Annelids. Some small portion of that evidence will engage attention here.

All major biological problems are embedded in huge masses of literature representing the attempts at solution of many investigators. Regeneration, because of its inclusion of so many more general problems, has a particularly voluminous literature. The restrictions of a single treatment impose the necessity of confining attention within certain bounds. Logically, the first consideration must deal with the nature and behavior of cells engaged in the regenerative process. Secondly, can the morphogenic properties of regenerative cells be controlled, and can the nature of the process be inferred from the nature of the action of the externally applied experimental influence? Thirdly, in how far are similar observations and controls discernible in regeneration in one of the higher invertebrates, the Annelids? Fourthly, do single cells, in this case Protozoa, exhibit the same sorts of organismic morphogenic controls as do the Metazoa?

THE HISTOLOGIC BASIS OF REGENERATION AND REASSOCIATION IN LOWER INVERTEBRATES

DR. W. C. CURTIS
UNIVERSITY OF MISSOURI

BEFORE proceeding, I should explain what I shall mean by "organismal" factors in regeneration as contrasted with "histological" factors. By histological factors I mean, of course, the changes whereby the various types of cells regenerate the new individual or part. By organismal factors I mean the physiological conditions in the organism as a whole, or in certain parts of the organism, that may be significant in relation to the histological and other changes of the regeneration. Thus, cell divisions, migrations, differentiations or dedifferentiations are histological factors; polarity, symmetry and metabolic gradients are organismal factors. These two aspects of the regenerative processes may be distinguished, however intimate may be their relationships in the final analysis. Regeneration can be studied from either of these aspects, at times to the disregard of the other; there can be no complete understanding of the process until we have adequate knowledge of what the individual cells are doing of themselves and under the influence of the masses of cells wherein the organismal factors in some manner reside. This complete analysis should be kept in mind as the goal, however distant its attainment may seem. Although my own concern has been principally with the histological factors of regeneration, I have never been unmindful of the organismal aspects of the problem.

When one studies the histological factors the role of the mass of cells is inevitably considered, since the mass of the regenerate conditions the places where cells divide, where they migrate, where and how they differentiate. When one studies the organismal factors, the cells can be forgotten for the time being, if one is thinking not of

cells but of regions of the organism in their relation to the whole. There are, in fact, many papers dealing with the organismal factors of regeneration, which might have been written in the absence of any knowledge concerning cells and in which the word cell may not even appear. This is not said in disparagement of such work; the investigator has fixed his attention upon this aspect of the problem and proceeded accordingly. Indeed, I think the organismal attack upon regeneration, as upon the problems of ontogenetic development, has been much the more fruitful in recent decades. Under existing techniques there are certainly more things one can do with an animal as a whole or with pieces of an animal containing many cells, than with its cells individually. Moreover, the histological changes are often easy of determination as compared with determination of the organismal factors conditioning such changes. But in the last analysis it will be necessary for the investigator to "know his cells" as well as his organism.

Certain of the histological data with respect to regeneration in the lower invertebrates may now be presented in summary statement. Since the Porifera have almost no specialized parts that can be removed and then restored, and since growth in sponges so overlaps with restorative processes, it is difficult to speak of regeneration in the Porifera in the same sense as in other phyla. Moreover, the restorative powers of many sponges appear to be limited, although some genera can be reproduced from cuttings and the development of gemmules in fresh-water sponges has certain resemblances to regeneration. The conspicuous phenomenon in sponges is the reassociation. Like the processes in sponges that can be called regenerative, reassociation seems to occur only in certain species. Hence, the problem of contrasting regenerative powers appears even in these lowest invertebrates. The histological study of reassociation indicates that dedifferentiation and subsequent redifferentiation into cells of other types do not occur. The

only cells that survive in numbers in the reunition masses are the choanocytes, which lose their collars and flagella temporarily but become normal choanocytes again in the newly forming mass, and the archæocytes, one type of which, the nucleolate cells, can form all the other cell types including choanocytes, while another type, which includes smaller and more active cells, forms only the new epidermal membranes. The cells of the epidermal membranes in the original sponge and the cells of the middle region seem to disintegrate and thus to have no part in the restoration. It is difficult to be sure what happens, but according to the late H. V. Wilson, whom we honor by this symposium, (1932, p. 166), "it has yet to be proven that sponge cells ever dedifferentiate into a regenerative, embryonic condition, although the dissociation phenomena in hydroids lend some support to the view that this is possible"; to quote again from Wilson, "the retention by cells of their essential nature, even after complete morphological dedifferentiation, is illustrated by the dissociated collar cells." In sponges, with their indefinite bodily organization and manner of growth, the histological aspect of reassociation is uppermost. There is not much to be made of the matter from the organismal aspect where the individual is so diffusely organized, yet there is a problem. On the histological side, cells that can be studied in fixed material from the reassociation masses and from the normal sponge, and living reassociation masses that can be followed day by day in what might be called "natural" tissue cultures, offer tempting possibilities for investigation. The limitations are the difficulties in interpreting the types of cells and their grouping in the sponge, even before dissociation, and the low level of sponge organization. Aside from the problem of reassociation, I may express the opinion that the dissociated cells and the reunition masses of sponges present material for the study of morphological and physiological problems that is not yet appreciated.

In cœlenterates, the histological changes of regeneration are not easily followed. The traditional conclusion

that the interstitial cells of such a cœlenterate as the Hydra represent an undifferentiated stock from which all the other cell types arise, both in regeneration and in reproduction and growth, has been questioned of late years. McConnell (1933) describes abundant mitoses in the epitheliomuscular cells. Strelin (1929), on the other hand, has found in studies upon irradiated material what he regards as a complete correlation between the occurrence and activities of interstitial cells and the power of regeneration in *Pelmatohydra oligactis*. Kanajew in three publications (1926) first concluded that the basis for regeneration was the transformation of interstitial cells into other cell types. His later study (1930) did not substantiate this finding; he then concluded that buds and regenerating parts were formed principally at the expense of neighboring differentiated cells, as shown by the transplantation of parts that had been vitally stained as well as by histological study. The relative potencies of ectoderm and endoderm in the regeneration of cœlenterates have been examined without conclusive results. Gilchrist (1937) has found that pieces of ectoderm from a syphistoma of Aurelia can regenerate complete new individuals; Papenfuss and Bokenham (1939) find that neither ectoderm nor endoderm alone can regenerate a new Hydra. The development of an effective technique of vital staining, of irradiation, of tissue culture, or the like, might lead to more consistent results if there be consistency in the phenomena.

The data are much more extensive from the organismal standpoint. The individual is simple in the cœlenterate but its differentiation is sufficient for the purpose. Corymorpha, for example, has been an endless inspiration to Professor Child. What has been said in general regarding histological *versus* organismal studies upon regeneration can be repeated with respect to regeneration in cœlenterates. If the organismal attack has shown greater possibilities under existing techniques, this does not mean that the histology of the regeneration need not

be known, nor that the fundamental explanations reside elsewhere than in the cells.

In nemerteans, the comprehensive investigations of Coe have laid a foundation for organismal studies. The nemertean tissue presents difficulties for the histological approach. Nevertheless, Coe (1934) has made something of the histological changes. With respect to the contrasting powers of regeneration in closely related species, he ventures only the comment that these "may be dependent upon differences in the extent of distribution either of the activating agent or of the regenerative cells."

The turbellarians, notably the planarians, are more favorable for histological study; and they have served as a perennial source of material for the organismal attack. For many years investigators of the histological changes have commonly agreed that the so-called "formative" or "regeneration" cells of the mesodermal region are the immediate source from which new parts arise during regeneration. The disagreement is concerning the origin of these formative cells. Some maintain that the formative cells originate by dedifferentiation, others that these cells are a persistent stock (*cf.* Fig. 15, Curtis and Schulze, 1934). It has also been maintained that the contrasting powers of regeneration in certain genera of planarians may be related to the relative abundance of formative cells whatever their origin.

In my own studies and those of my students this problem has been attacked by the technique of irradiation in the hope that the riddle of the parenchyma, as well as that of the formative cells, might be brought nearer to solution. My conclusions regarding the effects of irradiation with x-rays are supported by the studies of Weigand (1930) upon the effects of irradiation with radium in several species of planarians. Briefly, these results of Weigand and myself are as follows: It appears that exposure to x-rays and to radium inhibits regeneration in planarians, as shown by the reduction or by the absence of scar-tissue and by the proportionate restriction of the

regeneration. When such non-regenerates are examined histologically, it is seen that the formative cells are destroyed in numbers related to the degree of irradiation and of failure in regeneration. It is almost impossible to find any such cells in individuals whose regeneration has been completely inhibited. One observes in the fixed material that the formative cells show signs of disintegration soon after the irradiation and that they may have disappeared completely before other changes become apparent. The similar effect of such irradiation upon cells with embryonic potencies will be recalled. Observing that the formative cells have thus been destroyed in non-regenerates, it is only natural to conclude that the failure to regenerate is due in part, if not wholly, to the elimination of these cells, which are known to be of prime importance for the regeneration. The weakness of this conclusion that the failure to regenerate is conditioned, at least in part, by the destruction of formative cells lies in the fact that it has not yet been possible to produce planarians in which the power of regeneration has been completely inhibited and which continue alive and otherwise normal for a long period as in the case of the annelid Tubifex, noted in a subsequent paragraph. The planarian which has received an exposure of x-rays sufficient for complete inhibition of the regeneration dies some weeks later with external symptoms resembling those produced by various toxic agents. One must admit that there may be general physiological effects induced by the irradiation as well as the effect that is recognizable in the destruction of formative cells—that the inhibition may be only partially conditioned by the effect upon the formative cells. Also, it can be argued that the primary effect is of a general physiological nature which conditions first the death of the formative cells and later the death of the planarian as a whole. It can be argued as well, in the absence of experimental evidence, that the primary effect lies in the differential susceptibility of the formative cells to the rays and that toxic effects of the disintegrating material thus produced are responsible

for the general physiological changes which end in the death of the entire animal.

In an investigation intended primarily to determine whether *Euplanaria dorotocephala* shows a differential susceptibility to x-rays as to many other agents, Strandskov (1934) has examined the effects of x-rays upon planarian regeneration. He finds a differential susceptibility shown by planarians to various agents in accordance with the observations of Child and others. He notes, p. 585, that the planarians affected by the irradiation "regenerated in most cases some, if not all, the tissues that they had lost as the result of the effects of the x-rays," although he says he would not have expected this result on the basis of my observations (Curtis, 1928) which he cites. Again, p. 584, Strandskov states that, "whether or not 'formative cells' of the type described by Curtis are present in *E. dorotocephala* the writer can not state, but if they are, they do not seem to be especially susceptible to x-rays, at least of the kind emitted by the tube under the conditions given." In view of the well-known occurrence and functioning of such cells during regeneration in many species of planarians, it would be surprising if they are not similarly present and functioning in *E. dorotocephala.* The evidence of their destruction, following exposure to x-rays, may be easily observed by one who studies the necessary histological preparations. In a later paper Strandskov (1937), as stated in his summary, p. 20, seems to find more evidence for an inhibition of regeneration in relation to x-ray exposure, although "most of the irradiated pieces regenerated some tissue." He says further that "there is a decrease in the maximum degree of regeneration with each increase of dosage; also with each increase in interval between the irradiation and the time of the cutting of the pieces." Again, he states, that "although it was found that x-ray dosages can be given which will prevent pieces of *Euplanaria* from regenerating any tissues immediately following x-ray irradiation, no piece which had permanently lost the ability to regenerate was obtained and kept alive for any considerable length of time."

It seems to me that nothing in these results of Strandskov (1934 and 1937) is incompatible with the hypothesis that formative cells are responsible for the histological changes during regeneration, and that these cells, being more susceptible to the rays, are destroyed by the irradiation, as they are seen to be when the experimental material is examined histologically. Since varying exposures to x-rays destroy varying numbers of these cells, the cases in which the power of regeneration persists can be explained as related to the survival of a certain number of formative cells; restoration of full regenerative powers would be expected when a sufficient number of formative cells had arisen from these surviving cells by division. If formative cells arise by dedifferentiation as well as by multiplication of existing formative cells, the explanation of the restored power of regeneration is even more reasonable in terms of these cells.

A problem with respect to the formative cells of planarians is their apparent migration. Since the evidence for this migration rests upon the interpretation of series in fixed material, it presents the limitations inherent in such evidence. For example, one must consider the possibility that the pressure of contraction in the fixation of the worm may change cell shapes and thus produce in sections cell outlines suggestive of migration. If one could observe the formative cells moving actively in tissue cultures or when bits of the planarian are teased in a saline solution, the observations upon fixed material would be confirmed to this extent. I have never seen these movements in the living cells although shapes are observed like those found in sections. With respect to the evidence specifically: The scar tissue, or blastema that is visible externally, is composed principally of these cells which later differentiate into the new parts. Internally, a new pharynx forms in a similar manner from an accumulation of formative cells. However, there is no cell division, in these accumulations of cells, sufficient to account for the appearance of cells in such numbers. Mitotic cell divisions do appear in numbers in neighbor-

ing regions of the parenchyma; and between regions of cell division and cell accumulation the formative cells are frequently observed to be shaped as though in migration. The time relationship in the series of fixed specimens confirms this interpretation.

Some years ago, I hit upon a type of operation and subsequent regeneration that presents what I regard as a crucial test of migration by the formative cells and also a test of dedifferentiation in this instance. By a special technique, one fourth to one third of the free end of the pharynx is removed without other injury to the planarian. With the possible exception of the head region, or of the genital complex, the pharynx is the most specialized region of the animal; there seem to be virtually no formative cells within its boundaries. If dedifferentiation occurs to produce the blastema that eventually appears at the cut end of the pharynx, one might expect that this dedifferentiation would be clearly recognizable in the absence of neighboring formative cells. What happens is that nothing happens at the tip of the pharynx until formative cells arrive upon the scene by migration. The cut end of the pharynx remains a raw stump until the necessary formative cells reach this exposed surface, apparently by migration from the parenchyma of the body and through the length of the pharynx. From the blastema composed of cells assembled in this manner the new epithelial, muscle and other cells are formed.

The relationships involved in this series of events include formative cells in the parenchyma in such numbers as might be expected in view of the physiological state of the individual. For example, worms from stocks active in fission have many formative cells and much mitosis in these cells; food is also a factor. As noted, there are virtually no formative cells in the pharynx itself. After the pharynx is cut there is perhaps an increase in the number of mitoses among the formative cells in the region of the parenchyma near the anterior end of the pharynx, although this can not be determined with certainty; soon there appear formative cells shaped and

located as though migrating toward the pharynx. Next, there is a noticeable accumulation of these cells against a barrier-like group of muscle fibers which radiate from the lumen at the base of the pharynx and presumably function in retraction of the extended pharynx, or perhaps in the peristaltic contractions by which food is pumped through the lumen. Only when the formative cells have thus accumulated in numbers at the barrier does the migration through the pharynx begin. It appears that this movement occurs principally within a few hours, because worms showing maximum numbers of these cells within the old pharynx are not so readily found as worms showing stages of accumulation at the barrier, or worms with stages of differentiation in the blastema at the newly forming tip. The time at which any one stage occurs is somewhat variable, but the sequence is definite. One finds cells in numbers at the barrier, in numbers within the pharynx, or in numbers at the tip, never in numbers at all three places. Typically, in the earlier stages after the cutting there are many formative cells at the barrier and there are no such cells at the tip; later, there is a maximum number of these cells within the pharynx and lesser numbers at the barrier and at the tip; finally, there is a fully formed blastema with many differentiating cells at the tip and almost no formative cells at the barrier or within the older part of the pharynx.

The appearance of the newly arrived cells at the beginning of differentiation is suggestive. Cells are seen applied to the old epithelium of the outer surface of the pharynx and of its lumen as though becoming attached upon arrival and differentiating under the influence of the old epithelium and of exposure at the surface. As the blastema becomes covered with the new epithelium, muscle fibers, gland cells and parenchyma begin to appear. The only interpretation of this sequence of stages seems to be migration as indicated. It may be that less differentiated cells such as those of the gut dedifferentiate on occasion, in other parts of the planarian, after the manner described by Steinmann (1926 and 1937); in

this case of the pharynx, I conclude that the cells of this organ remain unchanged and that regeneration is initiated and consummated only by the formative cells after they arrive at the tip.

Understanding of what may be happening histologically is easier in the turbellarian than in the cœlenterate. The difficulties in the turbellarian are the questionable distinction between formative cells and the apparent syncytial structure of the parenchyma, and the possibility that dedifferentiation occurs. I have remarked that most of the investigators who have studied the histological changes in planarians have concluded that the parts formed during regeneration arise directly from the cells commonly known as the regeneration or formative cells, whatever may be the source of these cells. It may be noted also that such cells give rise to the germ cells and to other parts of the reproductive organs when these are formed, and that such cells can be recognized in very early stages of development; formative cells are, in fact, much like the late blastomeres of the embryo (*cf.* Fig. 15, Curtis and Schulze, 1934).

With respect to the histology of regeneration in annelids, to which attention has been devoted in my laboratory, some of the cases that have been described are perhaps as clear as any in the animal kingdom. In *Tubifex tubifex,* the neoblasts, earlier described by Krecker (1910 and 1923) and others, were studied by Stone (1932 and 1933) with the aid of irradiation. Exposure to x-rays inhibits the formation of these neoblasts and also the cell divisions of the ectodermal and endodermal epithelium that are necessary for part of the regeneration. The irradiated worms from which posterior segments have been removed heal the wound and form a characteristic stump but nothing more. Again it seems reasonable to suppose that the known effect of x-rays upon cells with embryonic potencies is the primary factor involved and that inhibition of the action of such cells or their destruction is primarily responsible for the inhibition of regeneration. I say "inhibition" of neoblasts as well as de-

struction, because neoblasts are not clearly recognizable as a cell type among the peritoneal cells of the septa on which they make their appearance in the regenerate. The histology of regeneration in Tubifex lacks one item that might be desired, since the neoblasts are not found in normal worms as a distinct cell stock; it is only that peritoneal cells produce neoblasts upon proper stimulation. In this respect the formation of neoblasts, though more spectacular, resembles the origin of regenerative cells from the ectodermal and from the endodermal epithelium of the Tubifex. With respect to securing an individual "castrated" against regeneration, the case of Tubifex is perhaps as good as can be expected. In Stone's cultures some of the irradiated individuals survived 147 days without regeneration, or as long as the non-irradiated controls. Other irradiated specimens were cut at intervals during comparable periods, but formed only a new posterior stump without regeneration. The controls, on the other hand, regenerated after each of several cuttings during such periods.

Our advancing knowledge regarding the histology of regeneration in the several phyla has been summarized in various publications during recent years by Goetsch (1929), Curtis (1934) and Stolte (1933). Difficulties of the kinds cited in the foregoing comments upon the cellular changes in lower invertebrates are encountered in higher forms. The recent work of Thornton (1938 and 1938) upon the regeneration of the forelimb of Amblystoma has interested me particularly. This investigator makes a case for dedifferentiation that I find convincing. One must admit from the standpoint of genetic and cytological theory that if all cells contain all the chromosomes and hence all the genes, and if differentiation is principally a matter of the cytosome, then there is good theoretical reason why dedifferentiation may occur. Also, there is abundant evidence supporting the contention that, in the course of development, time and place make cells what they come to be, as Roux contended years ago.

One thing that troubles me with respect to Thornton's work is the correlation of his results with the established fact that irradiation by x-rays prevents regeneration in these same salamanders. If dedifferentiation occurs and if there is no stock of cells with embryonic potencies, the explanation is not so simple as the mere destruction of such a stock. There seems to me need for a complete study of the histological changes of regeneration in these salamanders as they may be related to the known effects of x-rays upon the regeneration. Exposure to x-rays inhibits, or at least affects profoundly, the regenerative processes in many known cases from cœlenterates to vertebrates. Current work by DeBoer (1940) shows that the regeneration of feathers, like that of hair, is checked by x-radiations. What does all this mean histologically? When we have the same kind of an effect by the same agency in so many widely separated cases the action of similar factors is naturally assumed, as the working hypothesis to be examined critically while investigation proceeds. In this case the hypothesis first indicated is the destruction of embryonic cells and the disturbance of mitotic cell division, both of which are known to occur in cells with embryonic potencies, following exposure to x-rays and to radium.

To conclude, in addition to reviewing certain investigations upon the histology of regeneration in lower invertebrates, I have emphasized my conviction that knowledge of what individual cells do in the course of regenerative development is necessary for any comprehensive understanding of this process. Investigation necessarily begins with the gross morphological changes and proceeds on the one hand to histological study and on the other to study of the subtile physiological states of the organism as a whole or of its parts. So far as possible the data from these two lines of study should be correlated. The organismal phenomena present more points of attack with our present techniques and perhaps will always be the more significant. Yet it is my conviction that the ultimate answers lie with the cells.

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THE ENVIRONMENTAL CONTROL OF REGENERATION IN EUPLANARIA[1]

DR. OLIN RULON
WAYNE UNIVERSITY

Zoologists long ago recognized the remarkable capacity of pieces of certain planarians to regenerate or reconstitute into whole animals. It has been, however, only within the last three decades that an experimental analysis of the process has been undertaken in earnest and certain factors controlling regeneration have been brought to light.

Before discussing the subject of environmental control of regeneration in Euplanaria, it should be mentioned that a large number of experiments involving thousands of animals have shown that *E. dorotocephala* (as well as a number of other species) normally possesses a gradient in physiological activity which is highest at the anterior end of the animal and gradually diminishes posteriorly until it reaches the posterior zooid region. It has likewise been shown that while all levels have the capacity to regenerate a normal head and to reorganize into a complete animal, pieces of a certain size and from certain levels of the physiological gradient fail to do so because of other correlative factors present in the piece. Our problem deals with the control of such correlative factors as well as the direct control of regenerating tissues through the use of various environmental agents.

Let us now pass on to some of the pertinent observations and experiments in which *E. dorotocephala* has been the chief experimental animal.

The Head-Frequency Problem

Transverse pieces of *E. dorotocephala,* if allowed to reconstitute in well-water in the laboratory, may develop any of the following head-types if the size and level from

[1] Since the presentation of this paper Hyman (1939) has pointed out that the generic name *Dugesia* has priority over *Euplanaria.*

which the piece is taken is controlled (Child, 1911a, 1911b, 1911c, 1912, 1914a, 1914b, 1916, 1920, 1924):

(1) *Normal*—with triangular head, auricles at lateral margins and two symmetrically placed eyes.

(2) *Teratophthalmic*—with triangular head and auricles in normal position but with eyes showing all degrees of approximation to the median plane from two distinct eyes with pigment connected up to complete cyclopia.

(3) *Teratomorphic*—with head more or less rounded in outline and with auricles more or less anterior and showing all degrees of approximation to the median plane up to a single median auricle and with a single, or apparently single, median eye.

(4) *Anophthalmic*—with head rudimentary, without eyes and with or without a single median auricle.

(5) *Acephalic*—with head completely absent.

When the anterior zooid of 14–16 mm worms is divided into fourths (exclusive of the head) it is found that the most anterior (*A*) pieces regenerate almost 100 per cent. normal heads. In the next most anterior (*B*) pieces the number of normal heads is decreased while the number of inhibited heads and forms in which the head is completely absent is increased. Such inhibition in head formation is even more noticeable at (*C*) levels, while the most posterior (*D*) pieces may be almost completely anophthalmic and acephalic.

The frequency with which the different head-types appear at the different levels has been termed the head-frequency of that level for pieces of a particular size. In order to arrive at a value for the comparison of head-frequencies of different levels of worms of the same and different lots, the "Head-Frequency Index" has been devised (designated as "mean" by Child and Watanabe, 1935). This is arrived at by giving to each head-type a number which approximates its degree of inhibition (*Normal* = 100; *Teratophthalmic* = 80; *Teratomorphic* = 60; *Anophthalmic* = 40; *Acephalic* = 20). The actual number of each head-type for any particular level is then

multiplied by the number corresponding to its degree of inhibition and the sum of all divided by the total number of worms regenerating from that level. It can readily be seen that the head-frequency index of pieces from *A* level, which regenerates almost 100 per cent. normal heads, will approach 100 while the index of *D* level, which regenerates chiefly acephalic forms, will approach 20. Many experiments have shown that if the size of pieces from posterior levels is increased, the head-frequency increases, and if smaller worms are used, the head-frequencies of pieces involving equivalent fractions of the entire animal are decreased.

Under normal conditions there seems to be at least two different factors which determine whether or not short transverse pieces will develop a head. The first of these has to do with the antero-posterior physiological differential of the animal. When the anterior portion of the worm is removed a new head will grow on the posterior portion, but the rate at which the new head will regenerate will depend upon the level of section (Watanabe, 1935a). When the section is made at an anterior level of the first zooid the rate of head regeneration is more rapid than when the section is made at a more posterior level of the same zooid.

Although the rate of head development is different at different levels, all levels can and do regenerate normal heads. The fact that the various medially inhibited head-types do appear in short transverse pieces from posterior levels of the anterior zooid points to other factors in operation in short pieces which are absent or reduced when only a single section is made. The production of inhibited head-types in short transverse pieces seems to be due to some factor or factors arising at the posterior cut surface of the piece which may last for several days or during the early stages of tail formation. Evidence has accumulated which indicates that this head-inhibiting factor may be largely nervous in character (Buchanan, 1922, 1923; Child and Watanabe, 1935; Watanabe, 1935b)

and is probably conducted to the head-forming region by way of the ventral nerve cords.

Experimentation has shown that if the head-inhibiting factor be blocked or eliminated, or the head-forming cells near the anterior cut surface be given a start in the reconstitutional process (*i.e.*, by delaying posterior section for twelve hours or more), short pieces from any level may be caused to regenerate normal heads. It seems not unlikely that the reason short pieces from *A* levels regenerate normal heads while more posterior pieces of the same size regenerate inhibited heads or no heads at all is because the more active head-forming regions of the anterior pieces may break from the control of posterior inhibiting factors while the less active head-forming regions of more posterior pieces are unable to do so. But since we have no way for complete analysis, it may be that the head-inhibiting factor is stronger or more active in pieces from the more posterior levels.

THE CONTROL OF HEAD-FREQUENCY

Many different agents in the hands of a number of investigators have been used to increase the head-frequency in short posterior pieces. Such agents as dilute KCN (Child, 1916), chloretone, chloroform, chloral hydrate, ether, ethyl alcohol (Buchanan, 1922), caffeine (Hinrichs, 1924), strychnine (Miller, 1937), H-ion, carbon dioxide, methylene blue, formic acid, acetic acid, propionic acid, and butyric acid (Rulon, 1936, 1937, 1938) when applied immediately after section for a given length of time and in sub-lethal dosages have been found to increase the head-frequency. Mechanical stimulation (Child, 1920), supersonic vibrations (Wiercinski, 1939), and an abrupt change in temperature from 20 to 29 degrees C. (Behre, 1918) have also been found to increase head-frequency.

On the other hand, the head-frequency of pieces from anterior levels and of long pieces can be decreased by allowing the pieces to regenerate in dilute solutions of

metabolic end products, ethyl alcohol, KCN (Child, 1911a, 1912, 1916), certain anesthetics (Buchanan, 1922), and caffeine (Hinrichs, 1924).

Presumably an increase in head-frequency can be brought about either by blocking, or in some manner decreasing the head-inhibiting action of the posterior cut surface, by differential recovery of the head-forming region from the effects of general protoplasmic depressants (Buchanan, 1923), or by directly stimulating the head-forming cells near the anterior cut surface. A decreased head-frequency seems to result from either an increased effect of the inhibiting factor or a decrease in the activity of the head-forming cells near the anterior cut surface.

Animals of the same species and of the same size but from different stocks do not always yield the same head-frequencies for similar levels. Likewise, worms from the same stock vary considerably from time to time as regards head-frequency. Animals collected in the winter may show a head-frequency that is considerably different from those collected in the summer. At any one time worms of the same size but from different localities may vary as regards head-frequency (Rulon, 1936). Evidently the physiological condition of animals prior to section is important in determining regeneration in pieces after section. This difference in physiological condition must have been impressed upon them by their different environments before section.

In this connection it is interesting to note that a Japanese species from near Sendai resembling *E. dorotocephala* gave practically 100 per cent. normal heads in anterior and posterior pieces of one-eighth the body length or larger while only in shorter pieces did inhibited heads appear. Animals from what appear to be the same species but from a different region (Kyoto) show a head-frequency similar to that of *E. dorotocephala* (Child and Watanabe, 1935).

Worms in different physiological condition respond differently to various external agents after section. In

general, pieces from animals which show a relatively high control head-frequency react more readily to such agents as CO_2 and high H-ion and show a greater increase in head-frequency than do those pieces which show an extremely low head-frequency in the controls (Rulon, 1936). On the other hand, pieces (one-fourth the length of the anterior zooid) from animals with a high-control head-frequency treated with M/100,000 KCN for 72 hours after section show a lowered head-frequency at *B*, *C*, and *D* levels while *A* level is unaffected. Pieces from animals with a low-control head-frequency but with the same treatment show a slightly lowered head-frequency at *A*, *B*, and *C*, levels while *D* level shows an increased head-frequency (Rulon, 1938).

When intact animals are treated in the laboratory with certain agents before section, the head-frequencies of both tests and controls are altered when pieces from these animals are allowed to regenerate in various solutions after section. Animals, treated with high concentrations of CO_2 for 10 days prior to section and the pieces following section allowed to reconstitute in well-water (Rulon, 1936), show a slightly increased head-frequency at *C* and *D* levels, a slightly decreased head-frequency at *B* level, while *A* level is unaffected. When pieces from such CO_2-conditioned animals are caused to regenerate in carbonate-free well-water at a pH of 4.15 the head-frequency is much higher at all posterior levels than non-conditioned animals regenerating in the same test solution. Normally, an increase in the calcium concentration of solutions of high H-ion will antagonize the effects of the H-ion on the regeneration of heads in Euplanaria but if the animals are treated with CO_2 before section, only a partial antagonism to the effect of H-ion is caused by increased calcium. Pieces from CO_2-conditioned animals treated with .00032 M propionic acid for 96 hours after section have head-frequency indices of 100 for *A* and *B* levels, 99 for *C* level and 97 for *D* level. The untreated controls in well-water had indices of 100 for *A* level, 66 for *B* level, 25 for *C* level and 22 for *D* level (Rulon, 1937).

In contrast to the effects of CO_2 in increasing the head-frequency, it has been found that intact animals from stocks showing a high head-frequency treated for six days with M/100,000 KCN show a considerably lower head-frequency in posterior pieces when sectioned and allowed to regenerate in well-water. It may be of interest that this effect can partially be antagonized by allowing the pieces to regenerate in dilute methylene blue (Rulon, 1938).

The Control of the Gradient in Rate of Head Regeneration

It was pointed out above that any level in *E. dorotocephala* will regenerate a normal head if the posterior section is eliminated or the inhibitory factors arising at the site of posterior section blocked or decreased. It was also pointed out that the head regenerating on the posterior piece of a worm cut in two regenerates at a faster rate if the section is made immediately behind the auricles. The time for head formation increases as the section is made more and more posteriorly in the first zooid. This gradient in rate of head formation is one aspect of the physiological gradient so often demonstrated in these forms.

Certain experiments have been carried out testing the effects of environmental factors on the gradient in rate of head formation (Rulon, 1936). Pieces five-sixteenths of the length of the animal from just behind the auricles (*X*-pieces) and from the next posterior level (*Y*-pieces – five-sixteenths of the length of the animal posterior to *X* pieces) usually do not show the effects of posterior section and were used in these experiments. The rate of head regeneration is indicated by obtaining the mean time of first appearance of eyespots as seen under the compound microscope. In one experiment involving fifty animals, carried out at a temperature of 20° C. and from a stock showing a high head-frequency in the controls, it was found that the eyespots appeared on *X* pieces at approximately 3.6 hours before they could be detected on

Y pieces. In an H-ion concentration of pH 4.2 the rate of eyespot formation was delayed approximately 10.6 hours in *X* pieces and 6.3 hours in *Y* pieces. In other words, the difference in rate of eyespot formation for the different levels is practically obliterated by allowing the heads to regenerate under such inhibitory conditions. It should also be mentioned that with similar animals under the same conditions the head-frequency gradient is also practically obliterated.

In another stock showing a low-control head-frequency at posterior levels it was found that the *X* pieces regenerated eyespots approximately 13.7 hours before *Y* pieces. Treatment with H-ion at pH 4.15 inhibited both levels so that the difference was cut from 13.7 hours to approximately 8.1 hours. The head-frequency gradient of this stock under similar test conditions was somewhat flattened out but by no means obliterated. It appears in this last experiment that the physiological differential was so great between the different levels that it was only partially destroyed by the H-ion.

THE CONTROL OF BIPOLARITY

The production of bipolar forms, that is, those types which have regenerated a head at both ends of the section, is another example of the decrease or elimination of correlative factors within a piece by the use of external agents.

Bipolarity has been induced in pieces 1 mm in length from *B* levels and from the region of the fission zone in *E. dorotocephala* by increasing the H-ion of the solution in which the pieces regenerated (Rustia, 1925). The most effective agent yet used, however, in the production of bipolar forms has been strychnine (Miller, 1937). Pieces one-eighth of the length of the body in 16 mm worms from the region just posterior the mouth (*E* pieces) placed in M/100,000 strychnine for 16 hours and then returned to well-water for the remainder of the regenerating period yielded as high as 31.4 per cent. bipolar forms while no bipolars appeared in the control.

It has also been found that bipolars can be produced in this form in one-eighth pieces from *E* levels in well-water by delaying the time of posterior section. In one case a delay of 18 hours in making the posterior section resulted in approximately 26 per cent. bipolars. When pieces were submitted to the effects of M/500,000 strychnine as well as the delay of posterior section as many as 73.8 per cent. developed as bipolar forms.

The high frequency of bipolar forms from *E* level seems to be correlated with the fact that this is the region of the fission zone. The pieces from this region seem to be characterized by little physiological differential between anterior and posterior ends. This, together with the fact that strychnine seems further to decrease correlative factors, apparently permits both regenerating regions to become physiologically isolated from each other and each to become activated sufficiently to regenerate a head.

The Control of the Scale of Organization

Perhaps the most interesting effect of environment in the control of regeneration in Euplanaria has to do with the alteration and control of the scale of organization. It was found some time ago that pieces of Euplanaria not only regenerated a new head and tail but also underwent a complete reorganization of internal structures. It was found that such structures as the pharynx and mouth arose in the newly reconstituting form in definite spatial relationship to the head.

When a portion of the body posterior to the anterior zooid is removed and allowed to regenerate in well-water at 20° C., the piece regenerates a new normal head and a pharynx which is somewhat anterior to its mid-region (Child, 1915, 1924). If the piece is caused to reconstitute under certain inhibitory conditions the head is smaller and inhibited, with the pharynx appearing more and more anterior in the piece as the degree of inhibition is increased. If, on the other hand, regeneration is accelerated by increasing the temperature the scale of organization

is increased in that the head is larger and the prepharyngeal region lengthened.

The position of the various posterior organs seems to be correlated with dominance of the anterior end or the steepness of the gradient. If the anterior end has a high activity the length of the animal dominated and controlled is greater than when the activity of the anterior region is lowered as in the case of differential inhibition by temperature. The scale of internal organization seems to be controlled, at least in part, by environment in the action of external agents through the gradient system. If the gradient is steepened by differential acceleration or recovery, the scale is increased. If the gradient is flattened out by differential inhibition, the scale is decreased.

The effect of external agents in the modification of the scale of organization might be shown in another way (Rulon and Child, 1937). Short transverse pieces (one-sixteenth of the length of the animal) from the region just behind the auricles are from a region of high physiological activity, and, because of this activity, reorganization often takes place on a larger scale. Since the size of the piece is limited, the only part forming is that part which is most active in the complete animal. We therefore have a high percentage of forms regenerating from this region which are little more than heads since such short pieces regenerate a large head but little if any tail or posterior end when regeneration takes place in well-water under controlled laboratory conditions.

When similar short pieces are subjected to M/100,000 KCN for 72 hours and then returned to well-water the scale of organization is found to be decreased in many cases in that the number of tailless or partial forms is decreased while the number of forms with inhibited heads but normal tails is increased.

CONCLUSIONS

No developing organism is ever entirely free of the influence of environmental factors and the plasticity of

planarian tissues makes these forms all the more susceptible to environment and environmental changes. Inhibitory environmental agencies may act directly on regenerating tissues as in the case of inhibited heads in anterior or long pieces or they may act indirectly in the blocking or eliminating of correlating factors residual in the piece or arising at the site of posterior section.

Such factors as physiological dominance and isolation and such phenomena as differential tolerance, recovery, inhibition and acceleration may play a determining part in the type of regeneration of planarian pieces taken from a gradient system and already possessing, at least in part, the polarity of the parent. *E. dorotocephala* will regenerate a normal head at all levels and from short pieces of all levels if correlative factors within the piece be controlled. Such factors may be at least partially controlled by environmental influences imposed on the piece either before or after section. When the head is present it seems to arise in spite of the piece and on appearing tends to dominate to a certain extent the reorganization of the piece into a normal whole.

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ASPECTS OF REGENERATION IN ANNELIDS

LIBBIE H. HYMAN
AMERICAN MUSEUM OF NATURAL HISTORY, NEW YORK CITY

THE annelids are of great interest for the problem of regeneration because they are among the most highly organized animals capable of complete regeneration. Consequently, regenerative changes can be more easily followed than in lower, less differentiated forms, and the counting of segments can be used as a quantitative measure. Since the leeches are incapable of regeneration, the discussion confines itself to the oligochaetes and polychaetes. No new data are presented but the paper attempts to organize and correlate the known facts of annelid regeneration.

Among the annelids, as in the planarians, all gradations in ability to regenerate completely, occur. The word completely is used advisedly since practically every oligochaete and polychaete is able to regenerate posteriorly. It is then the question of anterior regeneration which is of most interest in annelids. In both the oligochaetes and the polychaetes this power is present in varying degrees. Among the lower or microdrilous families of oligochaetes, as the Aeolosomatidae, Naididae, and Lumbriculidae, almost any piece, even of very short length, with the exception of the body ends, regenerates completely. Among the higher or megadrilous oligochaetes, sporadic cases occur whose powers of regeneration are nearly equal to those of the microdrilous genera as the earthworm Perionyx (Gates, 1927). But in general, among earthworms only anterior levels are able to regenerate a head, and this ability ceases in the neighborhood of the fifteenth to twentieth segments. The least powers of head regeneration are seen among oligochaetes of intermediate taxonomic position, as the families Tubificidae and Enchytraeidae, which are practically unable to regenerate a head or, at best, can replace only the few most anterior segments when these are removed. Thus, among the

oligochaetes, power of anterior regeneration is related to taxonomic position only in a very broad way, for it is difficult to see any difference in grade of morphological differentiation between the Lumbriculidae with very high powers of regeneration and the Tubificidae with practically no ability to reform the head.

Moreover, among the polychaetes still less relation exists between taxonomic status and head regeneration. The errant polychaetes, universally regarded as the more primitive forms, as the nereids, eunicids, and phyllodocids, are in general incapable of any head regeneration at all, and die after decapitation. In another errant family, the Syllidae, all degrees of anterior regenerative power occur (Okada, 1928), and many members have almost unlimited powers of replacing the anterior end as Procerastea (Allen, 1921). But it is among the most modified and highly adaptive polychaetes, the tube-dwelling forms, such as the sabellids, cirratulids and serpulids, that unexpectedly great ability to regenerate the anterior end is found.

Clearly, gradations in regenerative power can not be explained as resulting from gradations in general morphological construction. Whether histological characteristics furnish an explanation is considered later. I may state at this point, however, that I do not believe any acceptable explanation has been found for the wide variations in ability to regenerate the head, which exist within a group of animals. That forms with regular modes of asexual reproduction, such as fission or fragmentation, have high regenerative ability is a truism; but why some annelid families regularly undergo fission and others of like morphology can not regenerate at all is not thereby explained. Again, several workers have attributed the regenerative powers of the tubicolous polychaetes to their liability to damage by enemies; but such a teleological view-point does not constitute an explanation.

The details of head regeneration reveal certain interesting facts. The ability to regenerate at the cut anterior surface approximately the number of segments removed

is exceptional. The best case of this kind is the syllid Procerastea (Allen, 1921), in which apparently the exact number of segments cut off anteriorly is regenerated; numbers varying from 9–31 were observed. According to Okada (1928), another syllid, *Syllis gracilis,* belongs in the same category, and Diopatra also is stated to regenerate as many anterior segments as are removed, although cuts did not extend beyond the thirteenth segment (Pflugfelder, 1929). In another group of forms, the number of anterior segments regenerated increases in general with the number removed up to a certain level, beyond which no further increase is obtained and the number replaced remains approximately stationary or declines as the level of section is moved posteriorly. A case in point is the earthworm Perionyx (Gates, 1927) in which if six or fewer anterior segments are cut off, the exact number removed is replaced; when more than six, the number regenerated increases with, but is generally less than the number removed and at no level are more than ten to fifteen regenerated. Similar conditions exist in Criodrilus (Tirala, 1913) which regenerates thirteen to twenty-three segments, mostly fifteen to eighteen, when twenty to thirty anterior segments are removed.

These are the less usual results. In the majority of annelids only a small number of segments regenerates at an anterior cut surface, regardless of the number removed, and in many cases this head regenerate has a distinctive morphology setting it apart from the rest of the body. Such annelids may be regarded as possessing a definite head or cephalic region, behaving as a unit in regeneration. Among the microdrilous oligochaetes, this cephalic region is usually obvious, being differentiated from the trunk region by the lack of dorsal setae bundles and absence of nephridia and chlorogogue cells. Here the number of head segments is definite and only this number is regenerated in anterior restitution. The number is three in Aeolosoma, five in the Naididae, except Pristina and its allies which have seven head segments, seven or

eight in species of Lumbriculus, three to five in the Tubificidae. Among lumbricid earthworms, the differentiation of a cephalic region does not show externally but is evidenced by the regeneration of a head of only three to five segments at an anterior cut surface. The most striking cases of cephalic organization are seen among the tubicolous polychaetes in such families as the Chaetopteridae, Sabellidae, and Serpulidae. Here the body is divisible into cephalic, thoracic and abdominal regions—each with well-marked morphological characteristics. The cephalic region bears various sensory organs and appendages and generally has reduced and modified parapodia; the thoracic region is also distinguished by various modifications of the parapodia and setae, and only the abdominal region bears typical parapodia.

It is characteristic of all such annelids with a definitely organized head region that regardless of the number of anterior segments removed, only this head region with its definite limited number of segments is regenerated. Consequently, the regenerated head may be attached to a quite different body region than normally. What happens then is the reorganization of the body region adjacent to the head into the normal postcephalic morphology. Among the oligochaetes the principal changes are the transformation of the intestine and the blood vessels; in tubicolous polychaetes, more striking alterations occur. In sabellids and serpulids, following a cut through the thoracic or abdominal regions, a head of three segments regenerates at the cut surface and this induces the reorganization of the adjacent old body segments into a thoracic region of typical segment number and morphology. Setae fall out, new ones appear, and the parapodia become transformed into the type characteristic of the thoracic region (Gross and Huxley, 1935, Berrill and Mees, 1936).

This reorganization of old parts to fit the regenerated head has long been known among lower invertebrates but is more obvious in annelids because of their greater morphological differentiation. The same phenomenon is seen

in coelenterates and flatworms where the head develops directly at the cut surface regardless of level, and intermediate regions must be reorganized from the old tissue. The important generalization which emerges from these data is that such reorganizations do not take place unless a head is regenerated. As phrased by Child long ago from studies on planarians, structures anterior to the level of section are not reformed unless a head or some approach to a head is first regenerated at the anterior cut surface. The only exception to this rule of which I know is one case in Sabella cited by Gross and Huxley (1935), where thoracic reorganization is said to have been initiated adjacent to the anterior cut surface without the occurrence of any head regeneration at that surface. One is inclined to believe there was some error of observation in this case, especially as no histological investigation was made.

It is evident that the head acts as a dominant or organization center from which some sort of influence extends posteriorly inducing the adjacent body region to transform into a normal postcephalic region typical of the species. The nature of this influence is of course debatable and various theories have been advanced. I am in accord with the conclusions of Child that the influence is transmissive in nature and the same type of explanation has been advanced by Berrill and Mees (1936) from their work on Sabella.

Ivanoff (1928), has attempted to explain on embryological grounds the very exact regeneration of a certain number of segments at the anterior cut surface in many annelids. It is well known that the trochophore larva often develops three segments and then pauses for a time in this three-segmented condition. Ivanoff believes these three larval segments constitute the three head segments of polychaetes like the sabellids and serpulids. In regard to polychaetes having a larger number of head segments, Ivanoff has attempted to show that the number of larval segments also corresponds; but in many of these forms,

the larva fails to pause at a definite stage so that the number of larval segments can not be determined with certainty. Chaetopterus larvae have nine larval segments, whereas the adult possesses a cephalic region of eleven segments. Since data on oligochaetes bearing on the matter were not available, Ivanoff undertook researches on the development of tubificids and lumbricids. He reached the conclusion that in these annelids also there is a definite number of larval segments, about six. Ivanhoff is thus of the opinion that when annelids regenerate anteriorly, they regenerate only the larval segments; but the facts already reviewed in this paper indicate that such an explanation, while no doubt containing some truth, can only be of rather broad application and can not be taken too specifically. According to Ivanoff the larval segments are those supplied by protonephridia and since these are evanescent, the head segments of annelids are devoid of nephridia. The transformation of abdominal into thoracic segments in the regeneration of tubicolous polychaetes is attributed by Ivanoff to the ingrowth into these segments of regenerated thoracic-type nephridia.

In the majority of annelids head regeneration is very definitely related to body level. In those forms which regenerate a head readily at almost any level and size of piece, such as naids and some syllids, it may be difficult to perceive any relation between antero-posterior level and reformation of the head. Generally, however, such relation is obvious. It may express itself merely as rate, in that the regeneration of the head is slower, the more posterior the level. More often a qualitative difference appears, especially when short pieces are employed. Thus in *Lumbriculus inconstans,* the typical head of seven segments is regenerated at any level in long pieces; but when short pieces are taken only the more anterior levels regenerate a normal head. More posteriorly the regenerated heads tend to be hypomeric with reduced number of segments and reduced prostomium and brain, and may take on a tail-like structure (Hy-

man, 1916). As already mentioned, there are many annelids in which the capacity for head regeneration ceases at some more or less definite level of the body axis. In lumbricid earthworms, the number of head segments regenerated declines as the level of cut recedes posteriorly and head regeneration ceases at about the fifteenth to twentieth segments. Similar conditions exist in the lumbriculid Rhynchelmis (Janda, 1918), the polychaete Clymenella (Sayles, 1936) and many syllids. Chaetopterus is remarkable in the abruptness with which head regeneration ceases; at the fourteenth segment, a complete head of eleven segments is regenerated, and at the fifteenth segment and beyond, no head regeneration whatever occurs (Berrill, 1928). Syllids may show a very abrupt decline in anterior regeneration at the level where the head of the sexual stolon forms. In some annelids, tails regenerate from anterior surfaces at levels posterior to those capable of regenerating heads.

A specific explanation for these facts is wanting. It is very difficult to see why a head can regenerate at, say, the tenth segment, and not at the fifteenth, when these two regions appear to be morphologically identical. Since any of these levels is capable of regenerating long tails of many segments, it can not be said that some element necessary for regeneration declines in numbers along the axis. It appears rather that some physiological condition associated with antero-posterior axiation is involved, as postulated by Child. It was intimated above that the regeneration of the head is a unique and more or less independent process which after it has gotten under way influences and controls the morphology of adjacent regions within a certain distance. Subordinate parts as those posterior to the level of section regenerate in correlation with antecedent parts, but for the initiation of the dominant, independent part, the head, certain physiological conditions must be established, primarily the breaking away from correlative influences. There

can be little doubt that the nervous system plays an important role in this escape from correlative influences. The nervous system is already organized for transmission in an antero-posterior direction. Because of this, an anterior cut surface is not subject to any extent to impulses from behind and can readily achieve independence, whereas a posterior cut surface is from the start subordinate to anterior regions by means of the nervous system. The assumption that the antero-posterior organization of the nervous system is best dedeveloped in anterior levels and becomes less and less effective as one proceeds posteriorly along the axis through the formation of postero-anterior paths would explain the general rule of antero-posterior decline in ability to regenerate a head.

A variety of data supports the belief that the central nervous system is of great importance in anterior regeneration. The better differentiation of the nervous system in annelids has made this group the favorite material for experiments of this kind. A number of workers have attempted to determine the role of the nerve cord in head regeneration in lumbricid earthworms by removing the cord from the segments adjacent to the cut surface. Recent experiments of this sort (Siegmund, 1928, Avel, 1930, 1932a, Kropp, 1933, Crowell, 1937, and Bailey, 1939) have confirmed earlier work that the head and brain may regenerate in the absence of the nerve cord at the cut surface. However, it seems to me that the emphasis has been wrongly placed in experiments of this type. In the majority of cases, a head fails to regenerate and, in general, head regeneration is much retarded and often imperfect in the absence of the main nerve cord. Hence the experiments really demonstrate the importance of the nervous system in anterior regeneration, in other words, the difficulty encountered by an anterior cut surface in establishing dominance without the assistance of the already established antero-posterior organization of the nervous system. Other types of experi-

ments testify to the morphogenetic action of the nerve cord. Thus Avel (1930) has shown that a head regenerates at the cut end of the ventral nerve cord when led to the body surface. A head regenerates at the anterior suture of a graft of ventral body wall containing nerve cord into the dorsal wall; if the nerve cord is absent, the graft simply heals in. Excision of a small piece of nerve cord of Lumbriculus induces the regeneration of a head in anterior levels, a tail in posterior levels (Haffner, 1928, 1931, Colley, 1931, Zhinkin, 1937), or of two outgrowths, an anterior tail related to the part of the worm in front of the wound, and a posterior head related to the part of the worm behind the wound. Cuts through the ventral cord of polychaetes may also induce head regeneration in appropriate body levels (Sabella, Gross and Huxley, 1935, Myxicola, Okada, 1932).

In lumbricid earthworms the ventral body wall also has a morphogenetic influence in head regeneration (Avel, 1932b, 1937). If dorsal integument at anterior levels is replaced by ventral integument including the nerve cord, two heads are regenerated, oppositely oriented. If ventral integument is replaced by dorsal integument, leaving the ventral cord in place, head regeneration is slow and feeble and often inhibited. If the ventral body wall of segments twenty-seven to thirty-seven (which are incapable of head regeneration) is replaced by a strip of ventral body wall from segments four to eleven, a normal head is regenerated following a cut through the grafted region; but if the experiment is repeated using dorsal body wall, a reduced head is regenerated in about 25 per cent. of cases. Various experiments indicate that the intestine has no morphogenetic action in anterior regeneration.

As already indicated, posterior regeneration is of less interest than anterior regeneration, but some points merit mention. Posterior regeneration is practically identical in macroscopic and microscopic details with normal growth. As we all know, chaetopod annelids

grow continuously at their rear ends by means of a growth zone in front of the anal segment; in this zone new segments are laid down in an antero-posterior direction. Only a very few oligochaetes and polychaetes have in the adult stage a definite number of body segments beyond which no increase occurs. Examples are Clymenella with twenty-two segments (Sayles, 1932) and Dodecaceria (Dehorne, 1924, 1932) with about twenty-five. It is stated that these polychaetes in posterior regeneration replace exactly the number removed. The polychaete Polyophthalamus (Stolte, 1929) is highly peculiar in that it does not regenerate posteriorly at all but merely heals the wound. Apart from these few exceptions and ectoparasites such as the Branchiobdellidae, any oligochaete and polychaete regenerates an indefinite number of segments from almost any posterior cut surface. As a rule the intestine fuses with the body wall forming a new anus, a growth zone is established in front of the anus, and new segments then arise as in normal growth. Such experiments as have been performed upon this point show that the length of posterior regenerate is greatest in anterior levels and declines posteriorly. This fact is in line with the suggestion that regeneration is related to the degree of antero-posterior organization, especially in the nervous system. In anterior levels where this organization is greatest the correlative influences acting upon the outgrowing tail will be strongest. In line with this explanation is also the fact that in some annelids, as earthworms and Lumbriculus, a tail may regenerate in place of a head at a cut anterior surface in posterior body levels. Wherever because of a decline in general antero-posterior integration, an anterior regenerate is unable to escape from adjacent correlative influences, it will develop under control of such influences into a subordinate part, or tail. The importance of the main integrative system, the nervous system, for posterior regeneration has been shown in several cases. Thus in the polychaete Nereis (Holmes, 1931) and the oligochaete Rhynchelmis (Zhinkin, 1936),

normal posterior regeneration does not occur if the nerve cord is removed in the segments antecedent to the wound. In Rhynchelmis following removal of a strip of cord, a tail may grow out from the cut posterior end of the cord.

It is generally true among oligochaetes and polychaetes that the cephalic region is unable to regenerate a tail unless the piece also includes a minimal number of trunk or thoracic segments. In tubicolous polychaetes often the cephalic plus the thoracic region is incapable of posterior regeneration or forms a tail in only a small percentage of cases. The exact number of body segments which must be left attached to the cephalic region before tail regeneration will take place has been determined for a number of annelids but no particular light has been thrown thereby on the matter. To say that the cephalic region is too differentiated to undergo the de-differentiation necessary for regeneration processes is only restating the fact in other words. Besides, we know that any part of this cephalic region will replace its anterior segments if these are cut away so that it is actually capable of regeneration processes. Some authors have attributed the phenomenon to the lack of nephridia in the cephalic region or the lack of other elements; but it has not been proved that the exact number of body segments which must be left attached to the head region before tail regeneration can occur exactly corresponds to the level where nephridia or other special parts or cell types begin.

It remains to consider the matter of the histology of the regeneration process. Annelids have been widely employed as material for the study of this question. In the early days, investigators were chiefly concerned with the germ-layer question, whether in regeneration new parts arose from the same germ layers as in embryonic development. Nowadays this question has faded from view and it appears further that the findings of the older workers were often in error. Consequently, only the later work is here considered.

In the study of regeneration processes in planarians and annelids, it is usually found that an indifferent type

of cell, variously termed formative cell, regeneration cell, or neoblast, plays an important role. In planarians, Curtis and his associates (1934) have suggested that regenerative power is directly dependent on the occurrence and number of these cells, without denying that other factors may also be concerned. They have shown that in genera of the Planariidae, such as Dugesia (= Euplanaria) with high regenerative power, the formative cells are abundant throughout the parenchyma, whereas in genera of the Dendrocoelidae, such as Procotyla, with poor regenerative ability, the formative cells are scanty. However, their own data show that formative cells do exist throughout the body length of Procotyla, including regions incapable of head regeneration, and that these cells take part in head regeneration as in other planarians. Attention may also be called to the fact that Procotyla annually with the approach of cold weather regenerates its gonads and a complex copulatory apparatus from these same formative cells. Clearly, Procotyla has an adequate stock of formative cells and these can regenerate a copulatory apparatus in a region of the body which is not able to regenerate a head. Again, it is the regeneration of the head which gives difficulty, and I believe this depends on other factors than the presence or absence of a certain kind of cell.

Among annelids, the data as a whole fail to support the formative cell hypothesis. In the various genera which have been investigated the histology of regeneration does not follow any one plan. Annelids in general possess a stock of indifferent cells, usually called neoblasts, which normally are found apparently inactive, on the septa and in strands along the nerve cord and muscle bands. The story of their role in regeneration differs with different genera. In posterior regeneration in Chaetopterus, Faulkner (1932) reported that the neoblasts multiply, migrate into the blastema, and differentiate into all the new parts of the regenerate including the epidermis. She gave a similar account of the formation of the new zooid in fission in the serpulid Filograna (1930). Probst

(1931) agreed with this account for Aricia, that in posterior regeneration practically all new parts originate from neoblasts, but in Owenia (1932) found an entirely different state of affairs. This polychaete has no supply of neoblasts; the new epidermis and gut epithelium come from the old ones, respectively, and all mesodermal parts arise from de-differentiated muscle. Most of the available accounts, however, especially in oligochaetes, report that the new epidermis, nervous system and other epidermal structures come from the old epidermis; the new gut epithelium originates from the old one, and mesodermal structures are formed from neoblasts.

From these varying accounts certain facts pertinent to the neoblast theory emerge. Thus, Aricia has a plentiful supply of neoblasts which form practically the whole of the regenerate, but is quite incapable of regenerating a head at any level. Faulkner's histological study of regenerating tails in Chaetopterus was done on cuts through the abdominal region; this region also can not regenerate a head, although as Faulkner showed, it is well supplied with neoblasts active in regeneration. The polychaete Polyophthalmus can not regenerate either a head or a tail at any level; yet according to Stolte (1929) has mesoderm cells of the neoblast type which perform the work of wound healing, so that, as Faulkner remarks, there is no histological reason for the lack of regenerative power of this animal. Although Turner (1935) found neoblasts participating in the anterior regeneration of the American species *Lumbriculus inconstans,* Ivanoff (1903) and Zhinkin (1932) agree that neoblasts are absent in head regeneration in the European *L. variegatus.* Krecker (1923) and Stone (1933) agree that neoblasts are not concerned in anterior regeneration in tubificids. In all of these oligochaetes, neoblasts are present in all trunk levels and give rise to mesodermal structures in posterior regeneration. From these facts it is evident that in annelids ability to regenerate a head is not correlated with the presence or abundance of neoblasts.

Recent work indicates that neoblasts may not be a defi-

nite cell type. Meewis (1934) found in Chaetogaster that each tissue has a supply of replacement cells, called basal cells by American workers, which reform that particular tissue in regeneration. The neoblasts are simply a stage in the alterations undergone by these replacement cells when they become active in regeneration. Similar facts have been reported for other oligochaetes by Krecker and Turner. Apparently then, the cells that accomplish regeneration are not undifferentiated embryonic cells but are already to some extent differentiated tissue elements and their transformation into neoblasts is a process of dedifferentiation.

It may be concluded that the central problem in annelid regeneration is the formation of the head since posterior regeneration is identical with normal growth. The regeneration of the head does not appear to be related to general morphological construction or to the presence or absence of particular histological elements, but rather depends on physiological conditions associated with antero-posterior axiation.

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CONTRIBUTIONS TO THE PROBLEM OF REGENERATION IN PROTOZOA[1]

DR. WILLIAM BALAMUTH
UNIVERSITY OF CALIFORNIA

I. INTRODUCTION

A. Material

THE Protozoa occupy a paradoxical position in the animal kingdom. These micro-organisms contain the solutions to fundamental problems of structure and organization within a single plasma membrane; yet relatively few significant experiments have been directed to the solution of these problems. The explanation does not lie wholly in the small size of these organisms, for some measure up to 2000–5000 μ; they can be cut precisely, and some have even responded to transplantation experiments. A more important reason seems to be the persisting conception of the *cellularity* of the Protozoa, with attendant emphasis on research in their reactions as cells rather than as organisms.

As Perty (1852) early pointed out, to be followed more completely by Dobell (1911), the body of a protozoön can not be compared to a metazoan cell, for one would thus be comparing a whole (the protozoan organism) to a part of the whole (the metazoan cell). From the point of view of the present discussion it would seem more promising to treat the Protozoa as organisms, subject to the same general factors of growth and individuation, of reconstitution and regulation, which operate in their differently organized metazoan descendants. Then the experimental aspects of protozoan development and reconstitution may be presented in the same general manner as has been done so successfully for the Metazoa.

[1] This report has been adapted for the purposes of the present symposium from a review now in press for the *Quarterly Review of Biology*. A complete bibliography covering protozoan regeneration accompanies the review.

B. Approach to the Problem

The problem of regeneration in Protozoa has been dealt with in two general ways. Most work up to the present time has been concerned simply with tracing the extent of regenerative capacity in the various groups. This approach is primarily descriptive, and can not give much insight into the nature of causal factors in differentiation.

A more fruitful approach involves experimental attempts to discover the mechanisms at work in regeneration. It is relatively simple to separate nuclear and cytoplasmic elements by cutting experiments, and thus to trace the relative roles of each of these important spheres of influence during reconstitution. No general unanimity exists regarding the nature of the controlling mechanisms. Some have stressed the role of cytoplasmic components in regeneration. Other investigators have placed emphasis on the concept of formative substances elaborated under the guidance of the nuclear apparatus. Relations of physiological dominance and subordination along major axes have been demonstrated in some forms. The relative importance of each of these lines of research remains to be seen.

Whatever may be the nature of the mechanisms, it seems clear that they must be closely related to those operating in normal reorganization processes (*e.g.*, in fission, encystment, sexual reproduction). This follows from the fact that both in normal and experimental reorganization of a given protozoön, the actual processes of simplification and re-differentiation follow the same fundamental paths, even when they prove circuitous. This condition lends added importance to the problem of regeneration in Protozoa. If one basic mechanism operates both in normal and experimental reorganization, then regeneration also partakes logically of the nature of a developmental process and its data should prove applicable in researches on developmental physiology.

C. Scope

In the present discussion some of the more representative Protozoa will be considered in the general order of advancing complexity. Wherever possible, some of the factors underlying morphogenesis will be emphasized.

II. Selected Studies of Protozoan Regeneration

The capacity for regeneration has been demonstrated in all the principal groups of free-living Protozoa. It seems advisable, however, to eliminate from the present review the flagellate forms (Mastigophora) and the exclusively parasitic Sporozoa. Few experiments have been performed on flagellates, and these have involved highly specialized forms not truly characteristic of the group (Noctiluca, Ceratium, Volvox, etc.). The data involving Sporozoa (only gregarines) are still inadequate, since these forms can not be cultured satisfactorily even under normal circumstances. This leaves two large groups of organisms, the pseudopodia-bearing Sarcodina and the ciliate Protozoa.

A. Sarcodina

(1) *Amebae.* Owing to their relatively simple organization, most work on these forms has resolved itself into testing the effects of enucleation on various vital processes, including regeneration. No essentially new data have been added since Gruber (1886) demonstrated that regeneration can occur in *Amoeba proteus* only in the presence of nuclear substance. Additional investigations have indicated that enucleation inhibits the assimilation of ingested food and results in a general metabolic breakdown. The qualitative unimportance of cytoplasm is indicated by the viability of as little as one eightieth of the cytosome in the presence of the nucleus (Phelps, 1926).

(2) *Shell-bearing Sarcodina.* The Testacea and Foraminifera present additional derived structures for experimentation. Verworn's work (1888) included a

comparative study of a representative of each group. Cuts on the testacean Difflugia never resulted in the regeneration of the shell, and this has been corroborated on numerous occasions. The foraminiferan *Polystomella* (= Elphidium) *crispa* reacted quite differently. Injuries to the body and shell were followed regularly by regeneration, so long as nuclear material remained in the fragment. Verworn reconciled this basic difference in reaction by pointing out differences in the normal developmental processes. Thus the testacean shell is formed in a single step at times of binary fission, while the foraminiferan shell adds to itself continuously during its lifetime. The formative mechanism seems to become irreversibly set at times of fission in the Testacea, while remaining labile and subject to experimental stimulation in the Foraminifera. The ability to regenerate seems to be a function of the normal developmental capacities in each of these groups.

(3) *Radiolaria.* These forms include some of the most highly evolved Sarcodina, exhibiting complex skeletal structures and a central capsule containing the nucleus. Verworn's investigation of the physiological significance of the nucleus (1892) led him to study *Thalassicolla nucleata,* a large peripylean type. He extended earlier work by showing that not only could isolated central capsules regenerate perfect organisms, but that homoplastic implantations of these into isolated extra-capsular cytoplasm insured viability of the latter (otherwise destined to degenerate). Additional evidence was given thereby to the nuclear role in control of viability, regeneration and regulation. This group should yield interesting results in such a field as heteroplastic transplantation.

B. Ciliata

The ciliate Protozoa have yielded the most extensive data bearing on regeneration. Complex locomotor and feeding organelles, a clear-cut nuclear dimorphism (macronucleus and micronucleus), and the presence of a

well-marked evolutionary series within the group have combined to permit various experimental approaches. The extensive data can be illustrated by referring to representative forms. It may be noted at the outset that the general regenerative capacity has proved excellent, so long as recognition is given to the occasional difficulty in recovering from the operative techniques.

(1) *Holotrichida.* This least specialized group of ciliates responds rather directly to merotomy,[2] judging from studies on fourteen genera of which Paramecium may be considered representative. Tartar (1939), author of the most recent and comprehensive experiments on Paramecium, has demonstrated that cutting results in the new-formation of organelles *in situ, i.e.,* at the cut surfaces. Missing oral structures are restored rapidly as a primary response, while regulation of body shape and the remaining regeneration of cilia apparently occur secondarily and in dependence on the former process.

Calkins (1911*a*) showed that the normal individuation process (binary fission) in Paramecium could affect the regenerative process. Thus merozoa often divide in the original center of the body to give two very unequal offspring, indicating the presence of a "fission zone" which becomes irreversible. Such instances of fission seem to be definitely non-adaptive, since the "bud" which forms is rarely viable.

The dimorphic nuclear apparatus in Paramecium includes an oval, centrally located macronucleus and one or more small micronuclei. Lewin (1910) and Schwartz (1934) found that the micronucleus was dispensable for the actual regenerative process, although experimental amicronucleate strains were not indefinitely viable. Apparently the macronucleus of these ciliates exercises more direct control of vegetative cytoplasmic processes than does the micronucleus.

Other holotrichous ciliates (Spathidium, Prorodon,

[2] Merotomy—a term used to designate the process of cutting a protozoön into fragments. (*Cf.* the term *merozoa,* used to designate the fragments produced by merotomy.)

Dileptus, Frontonia, etc.) present no discordant data, although it is true that none has been submitted to experimentation to a comparable extent. As a group the Holotrochida permit the generalization that there is a well-developed regenerative capacity in the presence of nuclear material, although the limited viability of amicronucleate strains leaves uncertain the precise relative importance of the micronucleus in vegetative activities. All the available evidence indicates that regenerating organelles (cilia, mouth region, etc.) arise *in situ* at cut surfaces rather than in connection with localized reorganization centers.

(2) *Spirotrichida.* These ciliates present more complex ciliary organelles and a more highly polarized organization than the Holotrichida. Within the group there can be traced a series from forms with an adoral zone of membranelles and simple body ciliation (Heterotrichina) through some in which the body cilia are strongly reduced (Oligotrichina) to a highly organized group possessing an adoral zone of membranelles and a restricted group of derived ciliary organelles (cirri) on the ventral surface (Hypotrichina). Correlated with this trend, the regenerative response tends to become more specialized.

(a) *Heterotrichina.* Stentor has been subjected to more regeneration experiments than any other protozoön (23 studies). Its trumpet-shaped body bears an adoral zone around the expanded anterior end; the macronucleus is a long moniliform structure and is accompanied by many small micronuclei. Between the time of Gruber's original experiments (1885) and the latest study (Schwartz, 1935) a well-rounded picture of regenerative capacity has been constructed.

Stentor responds readily to cutting experiments. Even relatively small fragments from the middle or posterior regions of the body can reconstitute a perfectly normal individual, providing only that a portion of the nuclear apparatus is present. The regenerative process itself

follows a precise, characteristic course. It is introduced by the appearance along the left side of the body of a dense, irregularly beating mass of short cilia. From this "reorganization zone" or "anlage," there differentiates a miniature adoral zone with oral and aboral ends; subsequent migration transfers this structure to the anterior end of the merozoön where it assumes the in-rolled orientation typical of Stentor. A rapid regulation of body shape and regeneration of body cilia complete the picture of cytoplasmic reconstitution. The nuclear phase of reconstitution involves contraction of the remainder of the moniliform macronuclear chain into a "balled" stage, following which an elongation and re-segmentation restore the vegetation condition. The behavior of the micronuclei is not so clear-cut; apparently they react by tending to multiply.

It is an interesting fact that the regenerative process in Stentor simulates very closely the normal process of asexual reproduction. In both instances a daughter adoral zone arises as a localized, immature anlage that subsequently migrates to its definitive position. Furthermore, the macronucleus undergoes an identical "balling" and "re-segmentation," and the micronuclei divide mitotically, in each instance.

The problem of the relative importance of the macronucleus and micronucleus for regeneration in Stentor has been subjected to experimental study (Schwartz, 1935). This German investigator undertook the direct approach of producing amacronucleate and amicronucleate merozoa and testing their responses. He found that, while amacronucleate individuals invariably died without evincing any ability to carry on digestion and assimilation, amicronucleate individuals regenerated perfectly. Indeed, from the latter Schwartz was able to culture amicronucleate clones which led a normal vegetative existence for more than a year. Verification of Schwartz's data will provide the first entirely satisfactory demonstration of the guiding role of the macronucleus in regeneration and the utter dispensability of the micronucleus in vegetative life.

The genus Licnophora is a highly specialized ciliate whose closest affinity has been sought by some among the Peritrichida (Uroceolaria, etc.), and by others (more recently) among the Heterotrichina. Work by Stevens (1903) seemed to indicate a very slight regenerative capacity, with replacement of missing organelles occurring only *in situ* and in the presence of both macronucleus and micronucleus. A more recent study (Balamuth, 1939) has disclosed, on the contrary, a broad regenerative capacity requiring the presence only of macronuclear material. More importantly, reconstitution of adoral membranelles always involves the formation and subsequent migration of a localized anlage, as in Stentor. This behavior entirely supports the view that Licnophora is a modified heterotrichous ciliate. In all, experiments have involved eight genera of Heterotrichina, and in none has any contradiction been leveled against the newer findings for Stentor and Licnophora.

(b) *Hypotrichina.* The hypotrichous ciliates comprise the end-group of the complex Spirotrichida. All traces of the primitive, uniform ciliation have disappeared, leaving only composite ciliary organelles (membranelles and cirri) localized on the ventral surface and a highly polarized body plan.

The genus Stylonychia has been the subject of several experimental investigations, of which those of Dembowska (1925; 1938) are most important. A remarkable regenerative capacity has been demonstrated; this involves the regeneration of an entirely new set of ciliary organelles and cytostome whenever merotomy removes one or more of these structures. The mechanics of replacement includes the formation of localized anlagen for the various groupings of organelles, followed by their gradual migration to their definitive positions *pari passu* with the resorption of the remnants of the old set of structures. This process resembles the mode of new-formation of organelles during fission, including even the site of the anlagen.

It becomes apparent that with advancing structural complexity in ciliates, reorganization becomes progressively more integrated. In the highest forms normal differentiation is clearly divisible into a primary morphogenetic response (formation of anlagen) and a secondary response that involves migration of organelles and general regulation. It is significant that the regenerative response follows this same course, even when it proves as uneconomical of materials as it often does in the Spirotrichida. This behavior becomes understandable if we postulate that there is only one mechanism whereby protozoan structures can be replaced; whenever this mechanism is set into operation it proceeds to completion.

No data are available for regenerating Stylonchia concerning the relative importance of the macronucleus and micronucleus. However, the nuclear apparatus clearly responds to merotomy by multiplying to restore the original pattern.

Study of certain hypotrichs has disclosed an apparent difference in regenerative capacity between "younger" and "older" individuals (age being based on the time interval between successive onsets of fission). Calkins' study of Uronychia (1911*b*) was the first in this connection. Briefly, he showed that with advancing age there was a markedly better regenerative capacity. Thus young individuals exhibited an irregular response and failed to complete reconstitution in the absence of both types of nuclei. Older individuals, on the other hand, regenerated consistently better and required the presence only of the macronucleus.

Apparently some gradual change occurs in the cytosome between periods of fission. The nature of this altered physiological condition should prove most important in analyzing the process of regeneration. Some light has been thrown on one phase of this problem by a recent study of two Russian investigators, Bauer and Granowskaja (1934*a* and *b*). Working with two hypotrichs, Gastrostyla and Oxytricha, they correlated the much higher

incidence of regeneration in older individuals with heightened respiration and macronuclear reorganization. Young experimentals reacted to merotomy by lowered respiration and macronuclear inactivity. This finding, if corroborated, may aid in explaining Calkins' results. The general relation between metabolic activity and the onset of the regenerative mechanism should be subjected to a critical study.

3. *Peritrichida.* This group is an offshoot from holotrichous forms and is distinguished by a characteristic circular arrangement of peristomal cilia and a general reduction of body ciliation. Little attention has been devoted to regeneration in the peritrichous ciliates, but at least one recent investigation is important in this connection. Summers (1938*a* and *b*) used a colonial form, *Zoothamnium alternans,* in which the component zoöids are arranged in a precise frond-like pattern. He discovered the existence of a well-developed regulatory mechanism in the growth and differentiation of colonies. Thus the removal of the dominant apical member of a colony resulted in the assumption of dominance by some dependent sub-terminal zoöid, and the colony proceeded to differentiate under this new leadership. Various experimental series confirmed this relation of a normally occurring dominance-subordination that apparently acts like the more widely known cases among Metazoa. Summers' data suggested also that younger colonial individuals are less rigidly determined than older ones, since the former exhibited more frequent regulatory responses.

In protozoölogical literature there are numerous references to the importance of physiological dominance and subordination during regeneration. It is well known, for example, that in such forms as Stentor and Stylonychia the presence of a remnant of the old adoral zone tends to inhibit the onset of regeneration of a new one. The general reaction obeys the expression, that the latent time before the onset of regeneration varies inversely as the degree of injury to the adoral zone. These and similar

observations have been interpreted as evidence of subordinating effects exerted by dominant regions. In this connection it is interesting to note that Child (1914) and later workers have demonstrated physiological gradients of activity along major axes in Protozoa. These are apparently analogous to those found frequently in Metazoa. Their importance has not been investigated in the Protozoa.

III. Question of the Mechanism Underlying Regeneration

As indicated at the outset, there has been no clear-cut demonstration of the causal mechanism involved in protozoan regeneration. Various stimulating suggestions have been made, and by examining some of the supporting data the present position may be briefly summarized.

In the first place, the processes of normal and experimental replacement of organelles seem to follow the same fundamental plan. The extent of regeneration is limited, in general, by the normal developmental potentialities (*e.g.*, see Testacea *vs.* Foraminifera). In each instance the fundamental problem is the preservation of species-identity, and all available evidence suggests a common mechanism in effecting the reorganization of nucleus and cytosome.

Control of the formative stimulus should be traceable either to the nuclear apparatus or to the cytosome, or possibly to some definite interaction between the two. The data collected thus far do not support the view of Loeb (1899) and others, which assigns the regenerative impulse entirely to the cytoplasm and regards the nucleus to be purely of subordinate importance.

We have seen that protozoan bodies contain physiologically dominant regions. It is necessary to test the possibility that the primary factor in both normal and experimental reorganization involves differences in metabolic activity. No physiological experiments of this kind have been conducted as yet.

The nuclear apparatus undeniably plays an important role during regeneration. Enucleate fragments never initiate regenerative processes, even though they may live and move for several days. Morphological evidence demonstrates that the nucleus (macronucleus of ciliates) undergoes complex form-changes in regenerating merozoa, and these usually begin before the onset of cytoplasmic reorganization. The extrusion of chromatin material into the cytoplasm has been frequently reported during reorganization, although this may have, and indeed need have, no connection with the transfer of specific substances. Chemical substances often remain unknown except for the demonstrable effects they produce.

The most convincing evidence supporting the importance of the protozoan nucleus in regeneration has come from the experiments on ciliates. It has been shown fairly conclusively in some of these forms that not only is nuclear material essential in regeneration, but more specifically the *macronucleus* is the controlling factor. Moreover, its influence extends to digestive and anabolic functioning, to judge from various investigations (*e.g.,* cessation of anabolic processes in enucleate merozoa), and therefore it seems to be the seat of more than one kind of secretory activity.

In this connection, finally, a recent study on an unicellular plant (the alga, *Acetabularia*) has pointed the way to a possible mechanism governing regeneration. Working with this extremely favorable material, Hämmerling (1934*a* and *b*) has shown that, as in the Protozoa, the presence of the nucleus is essential for regeneration. The method of "biological assay" of fragments enabled him to demonstrate a clear-cut gradient of morphogenetic impulse arising at the nucleus and proceeding along the heteropolar axis to the sites of new-formation. Perhaps the most significant experiment involved heteroplastic transplantation between two related species. The remarkable result was that the particular species characteristics differentiated in each instance according to the source of

the nuclei rather than the cytoplasmic mass in which the nuclei acted. According to Hämmerling, all the evidence supports the view that the effects are due to differences in concentration of formative substances, originating in the nucleus and acting at sites of morphogenesis. The orderly migration of substances may well be due to the influence of physiological gradients acting to set up local differences in accumulation.

Needless to state, it would be premature to claim the existence of such a mechanism in Protozoa. It is interesting, however, that as far as they go the observed facts seem to fit in with the mechanism suggested above. In any event, future investigations must take account of some of these unexploited lines of research, and to do so will involve greater emphasis upon the *Protozoa-as-organisms.*

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ADDITIONAL IMPLICATIONS OF REPTILIAN SENSITIVITY TO HIGH TEMPERATURES

RAYMOND B. COWLES
Biological Laboratories
University of California, Los Angeles

During the past year the writer has conducted extensive but as yet uncompleted investigations into the thermal responses of the supposedly heat-loving reptiles of southern California deserts. These have clearly indicated that far from being actually thermophilous animals, in the sense of favoring high temperatures, the reptiles conform to the usual thermal limitations of the metazoan protoplasm. This fact has led to a brief exposition of an hypothesis regarding heat as a factor in the history of terrestrial poikilotherms (Cowles, 1939). Subsequent consideration of the part which high temperatures may have played in the history of terrestrial vertebrates has led to the conclusion that additional discussion and elaboration of this subject may prove of interest.

Temperature and the Living Reptile

The optimum for most of the strictly diurnal species of lizards ranges from 37° C. (98.6° F.) to approximately 38° C. (100.4° F.). Species investigated in the field so far are, the chuckawalla, *Sauromalus obesus;* the desert iguana, *Dipsosaurus d. dorsalis;* the leopard lizard, *Crotaphytus wislizenii;* the short-nosed leopard lizard, *Crotaphytus silus;* the fringe-footed lizard, *Uma notata;* and the flat-tailed horned lizard, *Phrynosoma platyrhinos.* Optimum temperatures for the snakes lie between 31.6° C. and 33° C. for the nocturnal species such as the sidewinder, *Crotalus cerastes,* and the diamond-back rattlesnake, *Crotalus atrox.*

While Mosauer (1936) determined the approximate lethal temperatures for three lizards and one snake, the writer has found that the putative lethal temperatures

may vary widely depending on the criteria used in determining death. Due to the fact that respiration may cease several minutes prior to the termination of heartbeat, the usual criteria fail to give reliable results. There is a sharp increase in temperature the moment the cooling effect of respiration is lost, and from numerous observations it seems probable that the erratic breathing which takes place just prior to death may also influence the so-called lethal temperature. For these reasons it has been considered advisable to seek other criteria for the determination of thermal relationships at high temperatures, and investigations have consisted for the most part in a compilation of records of the temperatures at which the power of coordinated locomotion is lost.

When exposed to direct sunshine and high temperatures that prevail in spring and summer, snakes and lizards rapidly lose their capacity for well-coordinated locomotion, a situation which would normally bring about their immediate death under natural conditions. Using this point as the criterion for the practical lethal point, the method has the experimental advantage that rapid removal to a cool place will restore the animals to apparently normal conditions, thus affording an automatic check on accuracy, and a repetition of the experiment will produce almost identical results if not made too soon after recovery. For ecological purposes it may be assumed that this point, which has been termed the critical maximum, is the practical equivalent for the lethal temperature. When obtained by this method, field observations indicate an average critical maximum ranging close to 45° C. for the following diurnal lizards: the desert iguana, *Dipsosaurus d. dorsalis;* the chuckawalla, *Sauromalus obesus;* and the horned lizard, *Phrynosoma platyrhinos,* while the nocturnal snakes display a much greater sensitivity and are affected at temperatures ranging from 35.3° C. in *Sonora occipitalis* to 37.6° C. in *Crotalus cerastes.*

One of the most significant characteristics displayed

by both groups has been the considerable discomfort displayed at 3° C. above the optimum. Observations conducted over a period of three months during the spring of 1940 have verified these observations and also proved conclusively that the maximum temperatures which these lizards will voluntarily tolerate, even momentarily, lie at most only four degrees above the optimum. For any species so far maintained under observation, discomfort is so acute at temperatures between three and four degrees above the optimum that it is clear that prolonged exposures at these temperatures would inevitably result in serious damage.

Although serious discomfort and damage may result from exposures of three degrees above normal (optimum), these lizards submit freely to a lowering of the temperature to as much as 9° C. below their optimum. A further drop of 1° C., however, forces them to take refuge underground, but the capacity for locomotion apparently is not impaired seriously until a point is reached some twenty degrees below the optimum. It is also notable that while in hibernation they frequently sustain much lower temperatures. Even in midsummer, snakes and lizards may be maintained under refrigeration until their body temperatures drop to 1° C.–2° C. without suffering any apparent harm.

The remarkable degree of tolerance to cold exhibited by lizards and snakes from one of the hottest localities known and their more startling inability to withstand high bodily temperatures furnishes an intensely interesting study in adaptations of behavior. The physiological failure to cope with extremes of high temperatures has forced upon these animals a versatility in coping with the exigencies of hostile temperatures which is worthy of much study. Since it is possible that these forms may broadly represent prototypal thermal capacities, they have provided much interesting speculation on climatic conditions and the thermal ecology of the stem reptiles as well as the archosaurs.

Hypothetical Implications of the Past in Terms of the Present

That there is no infallible method of determining the temperature limitations of the archosaurs and their prototypes is obvious, but lacking any better criterion than that which can be found in the living forms of to-day, an imaginative venture into the past has been made on this basis in the hope that the following purely hypothetical proposals will provide interesting alternates to accepted ideas.

If the heat resistant capacity of the archosaurs and their prototypes can be accepted as resembling even our most thermophilous reptiles of to-day, it appears probable that they could have been no better able to cope with high temperatures than are modern desert lizards since these are species which have crowded the thermal ceiling of physiological tolerance. It is significant that the stem reptiles survived the coldest intervals of glaciation during Permian times and that modern types retain what appears to be a primitive capacity to withstand long periods of low temperatures without harm, even to remaining active above the relatively low temperatures of 27° C.–28° C. That some of the prehistoric reptiles were endowed with a similar capacity to maintain life through a wide range of temperatures seems reasonable from a consideration of the association of fossil plants and reptilian fossil remains, but it is highly improbable that any ancestral reptiles possessed a capacity to endure temperatures higher than those which are sustained by modern forms, a reasonably safe statement since the upper limit for the most heat-tolerant metazoans is supposed to be reached at 45° C.

Extant thermophilous forms may be considered as eurythermal types with an activity range of approximately 10° C., but their upward crowding of the optimum to a point just below lethal temperatures would place them in the stenothermal group so far as tolerance of any increase is concerned. The application of Leibig's

law here, "The factor for which a species has the narrowest range of adaptability limits its existence," seems to indicate conclusively that as between falling temperatures as a reason for the disappearance of the archosaurs or an increase above the optimum, the latter factor must be given the greatest consideration.

Without too close an analysis and with full recognition of exceptions, terrestrial vertebrates may be grouped roughly in a thermal sequence representing environmental or body temperature preferences. This can be accompanied by another generalization which will, however, bear further investigation, that the more primitive forms of each group require the lower temperatures. Some exceptions exist but, in general, the series ranges as follows:

Most specialized birds	Highest temperatures	
Less specialized birds	Lower temperatures	
Most specialized mammals	Highest temperatures	
Less specialized mammals	Lower temperatures	
Most specialized reptiles	Highest temperatures	Exclusive of the snakes
Less specialized reptiles	Lower temperatures	
Most specialized amphibia	Higher temperatures	
Less specialized amphibia	Lower temperatures	

Some familiar exceptions to this generalization may be recalled and a particularly obvious discrepancy may be found in the desert areas where the diurnal racers, although not considered as being specialized, are apparently the most heat-tolerant snakes. But while snakes are the most highly specialized of reptiles, their specialization has been such as to allow them to escape from the temperature fluctuations to which the less secretive, limbed forms are subjected and exceptions may be expected.

Investigations into thermal tolerance which are now being conducted show clearly that the snakes are incapable of sustained activity at either such low or such high temperatures as the lizards, and that, therefore, they should be relegated to a special category, possibly illustrative of the primitive, unaltered thermal requirements of ancestral types, probably perpetuated through early

adoption of nocturnality or a subterranean existence and consequent escape from the vicissitudes attendant on more exposed forms. In this connection Dr. G. L. Walls has kindly allowed the presentation here of some recently noted optical phenomena which provide independent substantiation of this possibility. His observations follow:

There is good evidence, from comparative ophthalmology, for believing that the ancient amphibians were strongly diurnal. On purely logical grounds, it is reasonable to suppose that the heavy-bodied Stegocephali would have sought the sunlight. Lacking enemies on land, they had no incentive to adopt nocturnality for purposes of concealment, and hence no need to forego those advantages of diurnality which accrue to a poikilotherm —the facilitation of locomotor activities and mental processes through the acceleration of metabolism.

The eyes of modern amphibians exhibit certain features which reveal that the diurnal forms—the frogs—have had uninterrupted diurnal ancestry since the amphibia arose from their piscine forebears. The most important of these features is the colored oil-droplet characteristic of the retinal cone visual cells. Associated firmly with diurnality wherever it is found among vertebrates, this cytological feature, once discarded, can never be regained. This is attested by the substitution for it of other color-filtering devices in diurnal snakes, diurnal geckoes, diurnal squirrels, primates, and insectivores —all of which lack oil-droplets and whose immediate ancestors were nocturnal, it being possible in every case to be certain that *their* ancestors, in turn, were diurnal and did possess colored oil-droplets.

The existence of colored oil-droplets in modern frogs is thus proof positive (to the satisfaction of a comparative ophthalmologist) that the Stegocephali, standing at the bottle-neck of amphibian phylogeny, were diurnal animals. They were probably more strongly so than the frogs, which are too small to dare tempt their many enemies and their high surface-volume ratio by exposing themselves too completely to the invigorating, but dessicating, action of the sun.

There are indications from the histogenesis of the amphibian retina that what capacity the frogs have for seeing in dim light was gained secondarily within the group; that, in other words, the frogs were once more strictly diurnal than they now are, which holds of course for the toads as well. The points involved are too complex, however, for lucid treatment in a brief letter.

If it were possible to do so, it would be exceedingly interesting to insert in the phylogenetic thermal arrangement the temperature preferences of the reptilian types from cotylosaurs to the dinosaurs, especially since there is reason to believe that the most logical position for the insertion should lie between that of the amphibia and the living thermophilous desert reptiles.

Although isolated species offer no safe criterion as to temperature relations because of possible specific modifications brought about by a response to specialized environment, it is none the less interesting to note the habitat of the nocturnal Sphenodon in a cool and damp environment. That the climatic factor may have been fully as important an item in survival as insular insolation is supported by the universal dependence of the crocodilians and most chelonians on an aquatic retreat where they can escape excessive heat.

With the limited information available, the association of thermal tolerance and phylogenetic arrangement can not be carried further than that given here, possibly due to the fact that even in the lower groups exceptional species have been able to take advantage of the acceleration of chemical processes at higher temperatures. The exigencies of adaptation for habitat and ecological niches, cause further confusion, but the close thermal relationship of vertebrate classes and orders strongly suggest a progressive adaptation to, and utilization of, higher temperatures. That all major groups have not been able to do so, suggests the possibility of a retention of phylogenetic limitations which may have been imposed by the environmental conditions pertaining during the origination of the group. It would appear logical to assume that environmental conditions existing at the time of development, and subsequently, might have left their imprint on the protoplasmic tolerance and so have provided us with some clues as to climatic conditions of the past and their influence on existing forms.

Although the evidence is by no means conclusive, it can readily be believed that in addition to alternating cold and warm cycles there may have been periods of excessive and continuous heat which have given rise to those forms which are now capable of taking advantage of the chemical acceleration which would accompany high bodily temperatures of around 37° C. While it is ordinarily thought that the tropical climate as now known may have

provided the requisite conditions, it appears probable that the tropics may actually be too cool. For instance, at Barro Colorado the mean annual temperature fluctuates between 26.7° C.–28.4° C. (80° F.–83° F.); in other words, approximates or even falls below the minimum temperatures to which the diurnal, heat-loving lizards will voluntarily submit, and furthermore falls roughly 10° C. below the optimum of these animals, or equally significant, 9° C. or more below that of the average homoiotherms.

Although the optimum, *i.e.*, self-selected body temperatures of tropical animals is not known, it is probable that the basking proclivities of many of the tropical reptiles represents an environmental thermal deficiency and an effort to elevate the body temperature above that of their air or water environment through a resort to insolation, and that only by this means can they achieve the required optimum velocities of chemical reaction.

Terminology

In the light of observations and experiments on desert reptiles and the resultant conclusions which are presented here, it becomes clear that more descriptive terms should be employed for the sake of accuracy and in order to clarify the nature of the poikilotherms as a group. They fall naturally into two categories as to methods of achieving optimum temperatures. The most convenient and descriptive categories result in a division into heliotherms and thigmotherms, the former receiving their heat chiefly through basking, the latter, chiefly through contact with substances in the environment—air, soil or water. Such a division naturally leads to the collective name "ectotherm" as an adjunct of or substitute for the inadequate characterization provided by the word "poikilotherm." The fallibility of the term is obvious since it is well known that many fish, and probably reptiles as well, live at a more constant temperature than many so-called homoiotherms.

The implication of the name "homoiotherms," which suggests the immutability of normal temperatures, is belied by many birds and mammals, and furthermore, both as a more suitable and logical contrast to the ectotherms, it is suggested that the warm blooded organisms may be conveniently referred to as endotherms. The employment of these categories is valuable not only in the study of modern forms but is also useful when applied to the theoretical conditions which may have existed in the past. This nomenclature is particularly helpful in visualizing and analyzing the possible conditions under which prehistoric organisms may have existed, or as a result of which they may have given rise to their successors.

Although the ectotherms are capable of producing some bodily heat, this factor apparently is of negligible importance throughout most of the environmental temperature ranges under which they normally operate, but it is realized that exceptions exist under conditions of great humidity with environmental temperatures slightly below the optimum, or where, as reported in incubation of the python, there may be a slight elevation of temperature due to a production of internal body heat.

If prehistoric vertebrates are thought of as being either heliothermic or thigmothermic, the question at once comes to mind as to the function which temperature may have played in the conditions under which the terrestrial habit originated.

It is known that ostracoderms were bottom dwellers and frequented shallow lakes or the littoral areas along these bodies of water. Therefore, the questions may be legitimately asked as to whether the habit of dwelling in shallow water may not have been a step toward heliothermy and that the subsequent emergence to land by later forms may not have been induced, in part at least, as a response to this upward step toward the achievement of advantages inherent in the greater velocities of chemical processes available at higher temperatures.

If the land habit evolved as a response to a gradual

drying up of the original habitat and the need for water, the emerging forms, theoretically, would have been obligated to face the dangers of dehydration as well as possibly excessive heat, or both, whereas if emergence were primarily due to the adoption of the shallow water-basking habit, alternating with short periods of land-basking, the transition from the aquatic to the fully terrestrial mode of life would have been a gradual and logical step, to be negotiated especially during times of high humidity and therefore with little hazard and many advantages.

An acceptance of this hypothesis requires the postulate that the climates of the Silurian, Devonian and Carboniferous were cool or temperate, thus encouraging or permitting a gradual adoption and dependence on heliothermy. Although this is apparently at variance with some interpretation of evidence obtained from the vegetation of the Carboniferous, it may be possible that plants of those "forests" require the cool, moist climates which, in general, are preferred by their modern descendants. In fact, the Bryophytes, Pteridophytes and Gymnosperms (apparently with some notable exceptions) suggest through their present concentration and distribution that they, as well as their ancestors, also may have required cool climates or habitats, whereas the more specialized as as well as more recent Angiosperms have invaded much hotter as well as dryer areas.

If heliothermy was the chief stimulant or one of the important stimuli involved in bringing about the terrestrial habit, it is probable that these early forms either possessed or soon evolved the radiation-absorbing dark pigments so characteristic of their more highly specialized descendants, but the capacity for thermal control through color change, so essential to most lizards of today, may not have evolved until later times.

Although no direct evidence bearing on this aspect is available, it is known that while many fishes possess the capacity for color change, it appears to be of value largely in concealment, there being very few fishes which are given to basking.

That the snakes do not possess a marked capacity for color change may be due to the fact that at least the desert species are characterized by an appreciably narrower range of temperature tolerance, a condition which has been determined by investigation into the thermal point of inception of locomotion as compared with the optimum. This trait appears to indicate that these forms, through their adoption of a nocturnal, subterranean or secretive habitat have either conformed to more primitive physiological limitations, or that through their selection of environments which are characterized by smaller changes in temperature, they have become incapable of the greater tolerances exhibited by their less specialized relatives. It is also true that their mode of locomotion renders them particularly susceptible to the lethal effects of high environmental temperatures. Their prone position subjects them to the full effect of combined radiation from above and direct contact with the heated ground from below. From the studies conducted so far it appears probable that snakes represent not only the most specialized morphology but the least specialized thermal adjustment.

If the ancient reptiles, like their nearest modern relatives, the crocodiles, possessed the dark pigmentation so essential for the forms of to-day, but lacked the additional faculty for color change, now an important protective device against both overheating and a too rapid heating, there can be little doubt that exposure to temperatures even a few degrees above those to which they were adapted would have led to their rapid disappearance or would have necessitated habit and habitat adjustments. Even if possessed of this protective device they would have been capable only of somewhat longer exposures, but at the same time they would have been seriously affected by a persistent increase in the intensity or duration of insolation.[1]

[1] Atsatt (1939) has pointed out that in *Xantusia vigilis* there is a peculiar reversal in color change responses with respect to heat stimulation. While most lizards are characteristically light when warm and dark when cold,

Although the geological record has provided glacial deposits, striae, alluvial and lacustrine deposits, etc., thereby providing direct evidence for marked lowering of temperature as well as a direct evidence for changes in precipitation, the evidence for slight temperature changes is entirely indirect and is available only through the distribution and types of fossil vegetation. Although there is ample evidence for periods of great aridity, the accompanying reduction in vegetation through which interpretations can be made often provide less obvious clues as to whether these arid and semi-arid intervals were characterized by high or low temperatures, and it is entirely possible that there may have been hot-dry and cold-dry intervals. In either case, the concomitant greater diurnal-nocturnal fluctuations would have imposed severe thermal changes on the ectothermotic organisms of those areas.

Although no direct evidence on these temperature changes is available, it is possible that a study of reptilian responses may suggest a logical hypothesis, and although it is possible that the archosaurs and their ancestors may have been characterized by temperature tolerances lying between those of to-day's urodeles and the desert reptiles, for the sake of argument they may be admitted as possessing tolerances as great as those of our most extreme forms of to-day.

That these ancient reptiles could have been eurythermal to temperatures much above 42° C. is entirely illogical. So far as known the protoplasm of all known vertebrates and most invertebrates appears to be subject

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Xantusia has reversed the change and is dark at high and light at low temperatures. This apparent aberration is a logical result of a high, dry habitat and the nocturnal, secretive habits of the animal. It is probable that this Xantusia is never voluntarily heliothermotic and that therefore it receives its heat supply from intimate contact with the walls of its retreat, or the air above ground, *i.e.*, by conduction rather than through radiation, and that the chief thermal occupation may be devoted to constant efforts to obtain the maximum available heat. It is highly improbable that it is ever faced with the problem of overheating.

to irreversible changes at temperatures of 45° C. for the Metazoa and possibly 50° C. for the Protozoa (Hesse, Allee and Schmidt).

In spite of the comparatively high temperature tolerance exhibited by such species as *Dipsosaurus d. dorsalis* and *Sauromalus obesus,* a few minutes' exposure to full sunshine on a hot spring day will induce a temperature of 44° C. and crippling within a few minutes' time. Such susceptibility in these species, probably among the most heat-tolerant of all known forms, indicates that even in the relatively mild condition of to-day there exists areas in which even the greatest possible physiological adjustments can not avert death as a result of insolation.

The larger reptiles, other things being equal, require longer exposures to produce the body temperatures that induce death, and both the desert iguana, and particularly the larger chuckawalla, are capable of extending their feeding activities later into the day than the smaller insectivorous species. This important extension of available feeding opportunities is partly a result of the favorable effect of their greater volume. From this standpoint it is conceivable that the tremendous size of many of the archosaurs, as well as the prevailing bipedal posture, were direct results of thermal limitation. The erect posture presents a smaller surface to insolation and removes the body from contact with the ground, while the great volume requires a longer period to heat as well as a longer period in which to cool off during the night. All of these signal developments can be readily explainable in terms of increasing heat and aridity together with the inevitable accompanying reduction in the size of xerophytic plants, and resultant decrease in available shade.

It would be expected that ectotherms subjected to increasing heat and reduced shade might seek escape in two major directions: (1) through an increase in size, which would eventually lead to a fatal thermal *cul de sac* in case of continued great increase in temperatures; or (2) through a reduction in size which would allow these

smaller forms to take refuge underground during the daytime to emerge at night.[2]

From recent study in the ecology of desert animals it is clear that one of the chief advantages of the subterranean mode of life results from the equable temperatures which are thus easily attainable in a super hot habitat, but even in deserts it does not provide complete escape from numbing cold. In addition to this advantage, underground retreats in hot deserts allow a selection of almost any desired temperature, these being readily available through penetration to the appropriate levels in the ground.

Small carnivorous and particularly insectivorous species of animals are provided with ample food supplies in these underground retreats, since burrows and caves are also used as refuges by all other small, therefore highly heat-susceptible ectotherms, a factor which explains the success of the almost universally adopted troglodidic habit. During periods of moderate temperatures, such as those occurring during the spring and fall of the year, the reptiles emerge from below ground, at first maintaining optimum temperatures by a constant shuttling movement to sun or shade alternating with periods of cooling in their underground retreats. During the autumn, surface activities are extended as temperatures drop from mid-summer maxima, *i.e.,* the animals become heliothermotic as soon as air temperatures fall below their optimum. Eventually all forms are once more driven to shelter by the long cold nights of approaching winter and the resultant cooling of the ground. The activities of prehistoric ectotherms may be envisaged as having been very similar under the same environmental conditions.

While fantastic, it may not be impossible that the great proliferation of armor and dorsal "fans" or "sails" (pelycosaurs and Stegosaurus, etc.) may have evolved, in part, as a device to avoid radiation of the tissues con-

[2] The absence of abundant sedges and grasses until Tertiary times is of great importance in this connection.

taining capillaries and other vascular therefore heat-conducting structures, from unmodified insolation. These would be of particular value over the region of head, neck and back as a protection to vital nerve centers from heat as well as enemy attack, thus serving a dual purpose.

As an alternative to the development of one or the other of these adaptations it is entirely possible that there may have been both annual north-south as well as similar major cyclic migrations. While seasonal migrations might have been possible for the more active forms, this recourse for the evasion of heat may not have been available to the larger, more cumbersome species.

The presence of palms and figs, corals and other warmth-loving organisms as far north as Alaska and Greenland during Cretaceous times seems to prove conclusively that these areas must have been tropical or subtropical in nature for part of the year, but the long nights and short days of winter must have produced conditions utterly unlike those of the equatorial tropics which are characterized by equal length of the days and nights. South of these pseudotropical areas, especially between 30°–35° north and south, the long days of summer must have produced intolerable heat, especially during periods of drought, and it is difficult to conceive of the equatorial tropics as other than intolerably hot during the short days, while remaining proportionally hot throughout the short nights.

Resort to migration to escape excessive heat may have produced a northward summer movement and a southward winter retreat, the latter presumably as a result of the long nights and by a possible contingent partial dormancy of vegetation. Since these annual seasonal movements may have been available only to the more agile species, it is conceivable that it may provide an explanation of the origin of the migratory habit which is so strongly developed in birds, but with the original north-south migration first adopted in order to escape heat. With the remission of excessive heat and a return to a

mild climate, diurnal fluctuations due to the changes in available daylight might perpetuate the movement. With a continuation of the climatic trend, cold weather would accentuate the movement once more, but throughout the entire cycle, seasonal movements would remain unaltered. It is even possible that high as well as low temperatures may explain the common transtropical movement.

The slow advance and retreat of the major nonseasonal cyclic changes would presumably have allowed a long range species diffusion (as contrasted with individual migration) to take place from the tropics northward and southward with the onset and development of excessive equatorial heat. While it is difficult to explain the extermination of archaic forms at the northerly and southerly limits of the continents where climates were presumably moderate, it is possible that the extremely long days, even though producing less intense insolation, in conjunction with the short nights, too short for effective cooling, may have been one of the factors instrumental in causing their death. This may be explained by a comparison with desert conditions, where black-bulb thermometers exposed in reptilian habitats frequently register as much as 85° C. (185° F.). Such conditions cause death in a few minutes at between 45° C.–50° C., but identical results are achieved with prolonged exposure to the more moderate early season sun and air temperatures which together produce body temperatures of between 45° C. and 50° C. Since such moderate but prolonged temperatures were by no means impossible during the Mesozoic times, no great stretch of imagination is required to visualize the ultimate destruction of ectothermotic animals. They may have been driven from equatorial regions by the high diurnal temperatures, and in retreating polarwards encountered, and may have been trapped by, less intense but greatly prolonged exposures to lethal heat. Supporting evidence for fairly high temperatures is to be found today in the geological records of the Argentine and Alaska.

Although it is difficult to explain the nearly simultane-

ous disappearance of the marine reptiles toward the close of the Mesozoic, the survival of crocodilians and chelonians indicates that some other factor than the direct effects of temperature may have been involved for aquatic ectotherms. Had these forms been oviparous there would be little difficulty in understanding their demise, but since at least some aquatic species are known to have been viviparous, it would be supposed that the young would not have been subjected to insuperable thermal hazards. As an alternate suggestion it may be supposed that some indirect effects of temperature changes may have altered the food supply, possibly either by a destruction of the prey, or by driving it to deeper, cooler water. The gill-breathing fishes could and might readily have retreated, while the air-breathing reptiles would have remained behind.

One of the most noteworthy aspects of the ectotherms as contrasted with the endotherms is the complete absence of effective insulating structures in any of the terrestrial forms, probably a condition which existed also in the ancestral prototypes. While internal insulation provided through layers of hypodermal adipose tissue is a common occurrence in the endotherms, whether endowed with surface structures or not, no such massive deposits are present in any terrestrial ectotherms. This characteristic appears to be directly connected with their dependence on outside sources for effective heating, and suggests the negligible value of such internally produced heat as is available. Apparently any attempts to conserve internally generated heat through external coverings so reduces the efficiency of the mechanism for heat absorption at moderate temperatures as to have prevented such a development. In this connection it therefore becomes essential to consider the circumstances under which the transition from ectothermy to endothermy may have come about.

Paradoxically, it seems logical to believe that the external insulating covering of birds and mammals

may have first originated as a device for protection from extremely high environmental temperatures, particularly as a defense against insolation. The total absence of any conspicuous insulating devices except color change in living reptiles, and the disadvantages which would result from either internal or external insulating devices, suggests that ancestral reptiles, birds and mammals, would have likewise suffered had these coverings been evolved under the conditions generally assumed to have been responsible for their initiation, that is, as a device to conserve internal heat in the face of a cooling environment. It is clear that external, insulating coverings would be hazardous for ectotherms since internal heat generation is ineffective, and such insulation would tend to occlude the needed heat of radiation.

If excessive heat played the part hypothecated here it is logical to assume that endothermism followed the evolution of types of ectotherms living under conditions of excessive insolation and having as one factor in their survival an effective mechanism of protection from radiation and a provision for heat occlusion. Certainly the lizards of our deserts, if endowed with either fur or feathers, would be capable of very greatly extending their hours of daylight activity during the hot season, simply through this protection from insolation. Furthermore, the low production of internal heat in these animals would enhance the value of such a covering under these conditions. Conversely, the possession of such a protection would be a severe handicap during cooler weather since it would exclude the necessary external heat. Considering the matter from another point of view, the possession of a coating of insulating substances which was capable of being erected so as to permit direct insolation of the skin during cooler weather, with a mechanism for lowering or closing up of the structures as the available outside heat dropped below that requisite to maintain the optimum, would extend enormously the length of the effective part of each day that could be profitably utilized by these animals.

While it is generally assumed that endothermism (homoiothermism), developed as a response to falling temperatures as a device to substitute body-generated heat for external supplies, this development would have required the simultaneous appearance of internal heat production and control mechanisms, operating in conjunction with the external insulating characteristics. While the development of dual interlocking or interdependent characters of this type could have taken place simultaneously it seems less plausible than that feathers and fur were originally evoked as a heat-excluding device, and that with the slow recession of the heat wave, followed by cooling nights and warm days, the prototypes of the birds and mammals may have resorted to partial basking made possible through the elevation of the feathers (and fur in the mammals), a particularly notable characteristic of the birds of to-day. Such behavior, followed by use of the same structures to occlude heat during the hottest part of the day and for the retention of externally acquired heat during the night plus an increase of the heat produced from within, thereby making it possible to carry over the optimum from day to day, may eventually have produced the types of endothermotic organism with which we are familiar to-day, *i.e.*, warm blooded forms with fairly uniform temperatures, maintained through heat production and conservation which has become possible through the presence of an ectodermal insulation of feathers or fur and subcutaneous layers of insulating fat.

SUMMARY

A presentation of an hypothesis to explain the great increase in size, the bipedal posture and the sudden disappearance of the giant reptiles, with an interpretation of their death in terms of the fatal susceptibility of present-day reptiles to temperatures in excess of 45° C. and the known thermal limit of 45° C. for most metazoans. While positive geological evidence for lethal high temperatures is not available, indirect evidence suggests the

possibility of extreme heat in circum-equatorial regions, which may have been comparable in intensity to the low temperature extremes which left their evidence in glaciation. If such an explanation is acceptable, the devices of feathers and fur may have evolved originally as a protection of skin and body from excessive insolation, rather than to conserve a probably negligible internal heat. The eventual development of effective internal heat production in conjunction with the previously supplied insulation, may have come as a response to the ebbing heat waves and thus have produced the comparatively efficient homoiotherms of to-day.

It is proposed that for accuracy and clarity the term "ectotherm" with the subtypes "heliotherm" and "thigmotherm" be substituted for "poikilotherm," and that "endotherm" be substituted for "homoiotherm." When considered from the viewpoint of the organic requirements of heat, there appears to be a definite progression away from the vagaries of thigmothermism through the adoption of heliothermism, with eventual stabilization by endothermism.

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SHORTER ARTICLES AND DISCUSSION

THE MASQUERADE OF LAMARCKISM

> Structure is after all the visible, highly mechanized end-stage of function, and our inability to detect the inheritance of an acquired character is probably due to the fact that its visible appearance is preceded in phylogeny by a period of many generations during which it is inherited only as a function associated with alterations of structure too subtle to be revealed by our present very crude methods of observation and experiment.—*W. M. Wheeler.*[1]

> No change has yet been induced in any chromosome mechanism which has any adaptive relations to the circumstances inducing it. . . . Hereditary properties always arise in advance of the uses to which they are put.—*C. D. Darlington.*[2]

INNUMERABLE records of critical experiments and authentic observations are available to support the general assumption that organs, individual organisms, species and biocenoses are capable of wide ranges of somatic and aggregative variability which are certainly specific responses to phases of nurture. Commensurate, excess or inadequate diet, extremes of heat or cold, moisture or aridity, exercise or lassitude, glandular substances, other and undetermined environmental factors have been shown expressly and specifically to modify the bodies or organs of animals and plants and their associations. The essence of Lamarckism is the conjecture that by some undefined means, or parallel and specific induction of the germ-plasm, perpetuation of these induced adaptive modifications is provided for through subsequent generations after the modifying applications have been discontinued.

The alternative to the Lamarckian hypothesis of use and disuse and parallel induction is undoubtedly the neo-Darwinian proposition that unspecified and insubordinated chromosomal changes and gene mutations supply the basic material variations. These, then, through hybridity, ecological selection and perhaps other means, determine evolutionary advancement, *status quo*, deterioration or extinction of species and biocenoses. Though far from completeness, this appears by wide agreement to be the most valid answer yet available to the inquiry concerning the causes of evolution. The vast, consistent, critically experimental data and logical inferences which have accrued mainly during the past

[1] "Essays on Philosophical Biology," p. 49.

[2] "Recent Advances in Cytology," p. 483.

forty years seem to be overwhelmingly acceptable as favorable to the neo-Darwinian conception and opposed to any scheme involving the Lamarckian hypothesis.

Among biologists, Lamarckism has become almost bereft of candid advocates. Each recent effort to give it experimental, or any sort of acceptable, support and restitution to respectability has brought contumelies upon the vindicator. As a scientific tenet it seems to be as dead as the dodo. Nevertheless, belief in and reliance upon the main features of this doctrine continue widely among husbandmen, parents, old-wives, medicine men and a few scientists. The ardent hope and ecstatic anticipation that specifically adaptive effects of the environment upon the soma may be demonstrated to endure somehow among the progenies for indefinite numbers of generations after the affecting agencies have ceased to operate holds on with persistent obstinacy. So inveigling and compelling have been the wish and hope of this supposition that its seductive allurements have impaired or obfuscated the equanimity of ratiocination of a number of philosophical paleontologists, psychologists and biologists.

Nevertheless, and granting that its intrinsic scientific merits are in question, or entirely untenable, Lamarckism, among other things, undoubtedly has validity and authority as a tradition, folk-lore or article of faith and hope. Although belonging properly in the realm of phantasmal science, this tenet may be no less potent than romance, creative fiction, constructive imagination, wishful projection or teleology. Probably to no one, or combination, of these, except possibly teleology, has sentient man taken recourse more congruously and efficaciously than to the folk-loric tradition of use and disuse and the inheritance of the characteristics thereby acquired. In such ways this popular belief has undoubtedly exerted effective influence in husbandry, eugenics, supplication, importunity, sagacious planning, and in most all affairs of man during his long, hard march up the rocky road of progress. In this sense the subject has been previously broached.[3]

Although it is assumed that not one iota of scientific evidence has been marshalled in support of the supposition, phenomena do occur in nature and among domesticated plants, animals and man which present something of the *semblance or appearance* of Lamarckism. Indeed, it does appear as if there is a tendency for the more permanent, heritable and adaptive morphological struc-

[3] AM. NAT., 61: 531–538, November–December, 1927.

tures and physiological properties to be closely parallel with, or accentuations of, the specifically environmentally induced somatic characteristics and functions, or *vice versa*. How do they become apparently so?

Mutations manifestly occur without specificity and particular reference to the surroundings, and completely insubordinate to the structures and functions which they condition whether they have adaptive capacities or not. The vast majority of all mutations are deleterious to the organisms in which they occur. Comparatively little account of these can be taken in nature because of their transitoriness and the lack of trustworthy records. They experience all degrees of survival from none at all to varying short lengths of time. Traces of them have been found in paleontology, some records have been made in the field, and the fact of their frequency amply established in many laboratories. Such unfavorable mutants are commonly ignored or forgotten also in the domesticated animals and man where the available records are often inadequate and undependable. Therefore, deleterious mutations are possibly of little, if any, general importance in evolution.

Other mutants, and perhaps in large numbers, are those that chance to conform somewhat better to the respective environs in which they occur. Most of these allow their bearers merely and miserably to suffer their living conditions and thus linger on the fringes of endurance and toleration. Indeed, it is likely that every organism at best experiences something of the element of mere toleration at times in, if not throughout, its life cycle. Examples could be cited by the hundreds, beginning with the turkey buzzard's and other carrion eaters' hard endurance, never enjoyment, of the ptomaines and other poisons in the putrefying flesh upon which they mainly subsist, and on to the most favorably conditioned bird or mammal. There are, then, just as indefinite numbers of mutants that happen better to adjust the individual organisms and species to their conglomerates of surroundings and relationships. Though perhaps fewer in numbers and still far from perfection, they are the ones that endure over the legions of those that are completely unfit and those that merely tolerate the environments for which they chance to be unaptly and precariously adapted. Under a given stress a mutant might be found to be beneficial, and in another complex of surroundings, to produce quite the opposite effect. There is, no

doubt, a shuttling back and forth in the value to a species of given mutants, depending upon the changes in the surroundings. Valuable mutants might logically be expected to spread rapidly and widely throughout a species, and harmful ones be limited, if they get any hold at all. Therefore, the few favorable ones should counteract the influence of a host of those that are not so propitious.

Somatic responses to changes in the surroundings exhibit extraordinary ranges of adaptive potency. Most organisms could not exist at all unless they were able to make somatic or physiological conformity to vast, frequent and often quite sudden changes in their environments. Such abilities are undoubtedly also products of mutations and selection. At no time must the features of the environment be so severe and inclusive as to surpass the abilities for adaptive somatic and aggregative responses of all the individuals in the widest range of the species. Among several habitats of grouse locusts which have been under observation for thirty years, not one has been fully available all the time. Most of them have been, due to drought, flood, oil, salinity or for some unobvious reason (possibly fungi or other disease), entirely unavailable one or more times. Fortunately, all of them have not been untenable at any one time. In connection with hybridity, the shifting of habitats from conditions of tenability to untenability and *vice versa*, over short and long stretches of time, gives almost any sort of mutant its chance, even though some of them may survive for only very short periods of time.

Now, if a sufficient number of individuals of a species can barely tolerate, through bodily and biocenotic responses, the strain imposed upon them by the progressively changing elements of the environment, times should come when chance mutations conforming with the nature of the somatic responses with which the organisms are already precariously meeting the stress will save the situation. If an environment changes gradually toward greater moisture, aridity, heat or cold or any conceivable complex, it may be logically expected that by relying upon the rare, fortuitously favoring mutants the species and aggregations may proceed in tolerably well-adapted relationship and parallel with it. Thus, tenaciously holding on by means of somatic variability and adaptability, remaining largely unaffected by the numerous inept, and profiting effectively by the apt, mutations, it is conceivable that individuals and populations may be directed specifi-

cally along the paths of, and parallel to, their gradually changing environments.[4] Paleontology is filled with incidents of such occurrences as well as with contingencies with which such mutations as occurred could not cope.

Furthermore, organisms, plants more than animals, themselves contribute to the changes in their surroundings, as has been observed for an indefinitely long time and lately confirmed by the ecologists. If the changes have extended trends and directions, and the stress is not too severe for the existing possibilities of somatic and aggregative responses, the rare, fortuitously favorable mutants, which are likely some times to occur, enable the organisms further and better to tolerate, and adapt themselves to, the very changes for which they are in some way or another responsible. These operations may be so subtle and complex that without rigid scrutiny it may *appear as though* the organisms are consciously directing their own destinies, or that some transcendental power, or entelechy, is involved.

If the foregoing inferences are cogent there is, then, merely the *masquerade, the semblance or appearance,* undoubtedly a legitimate, efficacious and beneficent folk-lore, but *not the actuality, of Lamarckism.* The concept of orthogenesis which has engaged the attention of evolutionists from Eimer to Osborn, and the support of some of them, has been closely allied with Lamarckism. The explanation of either of these doctrines need not be different from the other. A complex of environment which changes gradually in a consistent direction would carry, *by the processes herewith suggested,* the fauna and flora conformably along with it. Thus, there may be also the *appearance or semblance,* likewise congruent and justifiable, *but not the reality of orthogenesis.*

ROBERT K. NABOURS

KANSAS STATE COLLEGE

THE EFFECTS OF THE INJECTION OF ANTUITRIN G UPON A STRAIN OF HARELIP MICE

PHENOTYPICALLY normal individuals may show up (Normal Overlaps) in genetically harelip mice, as shown by Dr. Sheldon

[4] Since this paper was presented to the local seminar, February 15, 1939, there has appeared in the Quarterly Review of Biology, 14: 1, pp. 65–67 (March, 1939), Dr. G. F. Gause's review of "The Organism as a Whole," etc., by Professor I. I. Schmalhausen. I have been pleased and encouraged to note that the author had apparently, according to the review, already presented possibly a somewhat analogous view.

C. Reed, of McGill University. It seemed that the percentage of these Normal Overlaps might be influenced by internal environment or physiological actions. Accordingly it was planned to try a number of different physiological agents in an attempt to reduce the frequency of harelip, or at least to change its frequency, by increasing or decreasing the frequency of Normal Overlaps. Since harelip embryologically is due to the failure of the palatine and maxillary processes to grow and fuse together in a normal way, it was decided to try Antuitrin G, a growth hormone, first.

Harelip albino mice were obtained from Dr. Reed. Some of these were injected intraperitoneally with a daily dose of $\frac{1}{5}$ rat unit, and some with $\frac{1}{10}$ rat unit of Antuitrin G in .5 cc of normal saline. These doses were started just before mating and continued for ten days. This covers the period necessary for the maxillary and palatine processes to grow together and fuse normally. The controls were not injected in the harelip strain; however, non-harelip strains were also used as controls, and these had .5 cc of normal saline injected. Mice shown in Table 1 were used first as controls, and later as experimental animals, so that genetic variability was reduced. A standard ration was used and identical conditions maintained in so far as was possible.

The results of the injection of Antuitrin G into harelip mice is shown in Table 1. A dose of $\frac{1}{5}$ rat units (R. U.) of Antuitrin G had no effect on the frequency of harelip when its 29.4 per cent. was compared with the 30.2 per cent. of the control; however, the dose of $\frac{1}{10}$ rat units seemed to increase the percentage of harelip as shown by the 16.5 per cent. increase. It would

TABLE 1

EFFECTS OF THE INJECTION OF ANTUITRIN G ON A STRAIN OF HARELIP ALBINO MICE

	No. litters	No. individuals	No. harelip	Per cent. harelip	Mean litter size	Mean individual weight
Control	13	63	19	30.2	4.84 ± .60	1.33 ± .065
Experimental 1/10 R. U. ..	5	30	14	46.7	6.00 ± .76	1.11 ± .068
Experimental 1/5 R. U. ...	4	17	5	29.4	4.25 ± .43	1.27 ± .048

seem that this dose caused many possible Normal Overlaps to give phenotypic expression to their genetic make-up. Inconclusive figures seem to suggest that a dose of $\frac{1}{5}$ rat units decreases the

litter size, while a dose of $\frac{1}{10}$ rat units increases the litter size as compared with the control. The individual weights of the new-born mice is lowered in each case by injections, and is lowered most by the $\frac{1}{10}$ rat unit dose. Dr. Reed has shown that large litters have an increased percentage of harelip and the figures given in Table 1 also suggest a correlation in this respect.

There was no clear-cut change in the percentage of the different types of harelip (dextral, sinistral or bilateral types), although there is a suggestion that the bilateral type and left type may be increased relative to the right type by the injections of Antuitrin G.

Three experimental females had double parturition with more young being born in one, two and four days following the first-born. Many premature mice also resulted from these injections. Trials on non-harelip strains of mice produced neither of these effects. The gestation time was reduced in the experimental mice to an average of 17.3 days, with a range of 16 to 19 days.

These strains of harelip mice have been lost, and since the author would like to continue this experiment and try many other physiological agents, he would appreciate it very much if any one who might have harelip and cleft palate show up in his colony would get in touch with him. This condition showed up originally in the Bagg albino strain.

Acknowledgment is given to students who have worked on this problem under the direction of the author: Frank Fuller, Ronald H. MacDonald, Harold Doyle, Robert Woods and Lyle Hill.

L. C. GLASS

UNIVERSITY OF IDAHO

THE DECREASE IN WEIGHT AND MORTALITY IN DEXTRAL AND SINISTRAL INDIVIDUALS OF THE SNAIL, *FRUTICICOLA LANTZI*

THE majority of species of terrestrial and watery snails are dextrally coiled. The recessive sinistral individuals are relatively rare. It is generally believed that dextrality or sinistrality of coiling is not associated with any physiological advantage or disadvantage of the individual bearing it. There are some preliminary suggestions, however, concerning the unequal fitness of both types. On the one hand, the sinistral mutants usually occur in small isolated colonies, often separated by a natural barrier from the area of the basic dextral form (Ludwig 1932, 1936).

The distribution of sinistral specimens over larger territories appears to be impeded by their ecologic weakness. On the other hand, some physiological observations suggest that while in the individual of the typical coil the steric configuration of organic substances participating in the determination of twist might well conform to the levorotatory steric configuration of the other constituents of protoplasm, some enzymatic discoordinations might be expected in the organisms of the inverted individual (Gause, 1939). The purpose of this investigation is to compare under controlled laboratory conditions some physiological characteristics in the dextral and sinistral individuals of the same species of snail.

The terrestrial mollusk *Fruticicola lantzi* was collected in an isolated mountainous colony in the region of Alma-Ata (Middle

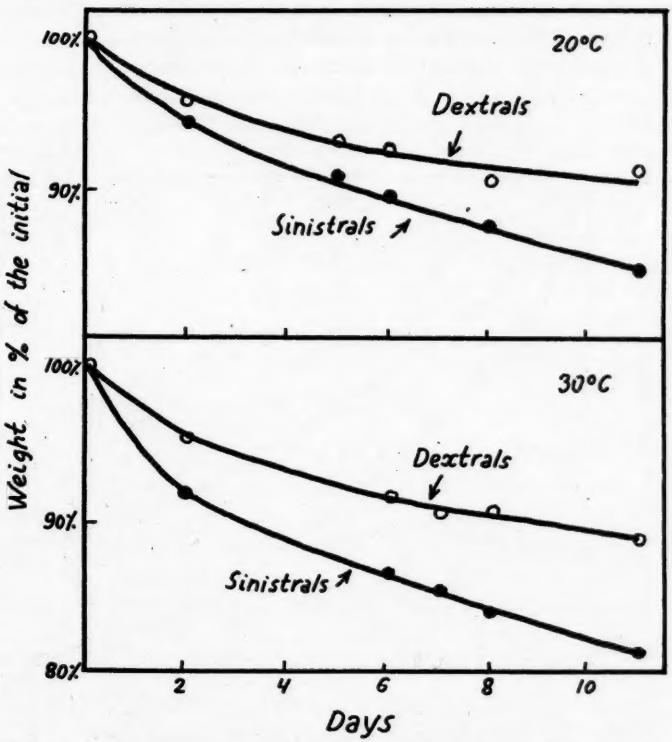

FIG. 1. The average curves of the decrease in wet weight in starving young snails *Fruticicola lantzi* at different temperatures.

Asia). Both dextral and sinistral individuals occurred together at the time of observation, and were apparently bred under perfectly identical geographic and ecologic conditions. The material was not easily obtainable, and we studied altogether 300 individuals of both types in the work extending over a period of two years.

The snails, obtained by parcel post from Middle Asia in a state of estivation (into which they very easily lapse in case of deficiency of moisture), were placed for reviving into a big moist chamber, and during several days before the experiment were fed on carrots. Then the snails were numbered and weighed individually on the analytical balance. Before every weighing each snail was carefully wiped with absorbent cotton.

In two series of experiments the decrease in wet weight in dextral and sinistral starving individuals was studied. The first series of experiments was conducted with young snails (diameter of the shell 10–11 mm). Ten dextral and ten sinistral individuals were placed in each moist glass chamber, in which there was nothing but a small amount of water. In all experiments snails were daily transferred into a new clean chamber. One chamber

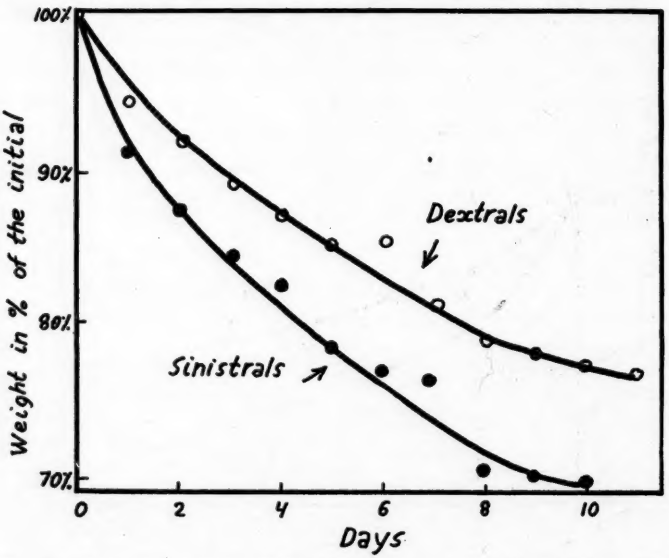

FIG. 2. The average curves of the decrease in wet weight in starving adult snails *Fruticicola lantzi* at 18° C.

was kept at 20° C. and another at 30° C. The change of wet weight of snails in these experiments represented a smooth gradual decrease. Fig. 1 shows that at both temperatures sinistral individuals lose in wet weight more rapidly than the typical dextral forms.

In the second series of experiments the decrease in wet weight in the starving adult snails (diameter of the shell 18–19 mm) in big moist chambers was studied. Twenty six dextral and twenty six sinistral individuals were kept under observation. Before death the enfeebled starving snail usually can not retreat into its shell, and such snails were no longer weighed. Fig. 2 makes it evident that the sinistrally twisted starving individuals decrease in wet weight more rapidly than the dextral snails.

In order to eliminate possible fluctuations in water content of the snails, the previous data collected in the summer of 1938 were extended in 1939 by measurements of the decrease in the dry weight in starving adult snails of the fresh collection. Both dextral and sinistral snails were combined into pairs of identi-

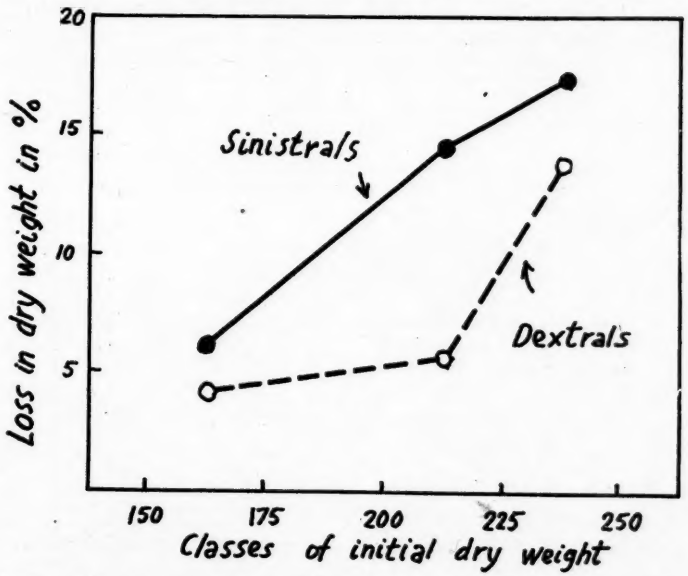

FIG. 3. The decrease in dry weight in dextral and sinistral starving adult snails *Fruticicola lantzi* belonging to different classes of initial dry weight in milligrams.

cally twisted specimens of approximately equal wet weight. The dry weight of one member of the pair was immediately determined, while the dry weight of another member was measured after a five-days starvation in a moist chamber at 25° C., and these two weights were later compared. For measurement of the dry weight the shell of the snail was carefully eliminated, and the remaining body was dried at from 70 to 75° C. for eight hours. Seventy four dextral and forty eight sinistral individuals were used in this investigation. Fig. 3 shows that sinistral individuals of all initial weight classes lose in the dry weight much more rapidly than the dextral individuals of the same initial weight classes.

The records of mortality in populations of starving snails have shown that in sinistrally twisted forms the mortality rate exceeds that of the dextrally twisted ones. In the first experiment with fifty dextral and fifty sinistral specimens, the mortality on the fifth day attained 27 per cent. and 40 per cent. and in the second experiment 42 per cent. and 54 per cent. correspondingly. As the sinistral snails for the maintenance of their living burn up per unit of time a comparatively larger amount of food reserves, they consequently expend their available food resources more quickly and die off more rapidly than the dextrally twisted forms of the same species.

G. F. GAUSE
N. P. SMARAGDOVA

INSTITUTE OF ZOOLOGY,
UNIVERSITY OF MOSCOW

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